APRIL 1984

003958

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Transmittal of Disulfoton (Di-Syston)® Registration Standard.

TO: Amy Rispin, Ph.D.
Science Integration Staff
Hazard Evaluation Division (TS-769C)

and

George LaRocca, PM#15
Registration Division (TS-767C)

FROM: George Z. Ghali, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Christine F. Chaisson, Ph.D.
Section Head, Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

With this letter, we are submitting the Toxicology Chapter of the Disulfoton Registration Standard. The Toxicology Chapter includes the following:

1. Disulfoton Policy Discussion.
2. Generic Data Requirements for Disulfoton.
3. Summary of the Evaluated Data.
4. Data Evaluation Records.

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The Policy Discussion Section emphasized the need for a tolerance reassessment based on the nature and magnitude of plant residues, taking in consideration the structural association of Disulfoton and other OP's. The susceptibility of infants in relation to existing tolerances was also discussed.

Because data requested by Toxicology Branch from RCB and EAB were not available at this date, reassessment of tolerance and the assessment of the hazard to workers or hazard from possible groundwater contamination are not considered complete.

003958

# DISULFOTON REGISTRATION STANDARD

## Toxicology and Human Safety

By

George Z. Ghali, Ph.D.
Toxicology Branch
Hazard Evaluation Division
US Environmental Protection Agency

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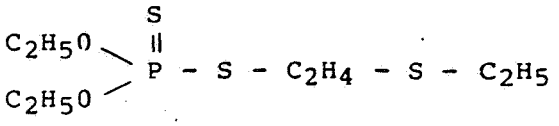
## INTRODUCTION

Disulfoton, also known as Di-Syston® (0,0-diethyl-S-[2-(ethylthio)-ethyl] phosphorodithioate) is a broad spectrum systemic organophosphorus insecticide/acaricide, developed in 1956 by Fabenfabriken Bayer, A.G., Leverkusan, Germany, and manufactured in the United States by Chemagro Corporation.



Disulfoton

The estimated annual production of Disulfoton in the United States is 5-7 million pounds, most of it is available for domestic use. Disulfoton is registered for use on a broad spectrum of food and non-food crops, in addition to numerous ornamental plants. The primary uses of Disulfoton are on cotton, sorghum, and wheat. These uses account for about 70% of the total use of Disulfoton.

Disulfoton is formulated into granular formulations containing 0.35 to 15% of the active ingredient, or emulsifiable concentrates containing 6 to 8 lb. per gallon. Granular formulations containing greater than 2% and emulsifiable concentrates containing 6 pounds per gallon or greater are considered as restricted pesticides. Only granular formulations containing less than 2% active ingredient are available for commercial distribution.

Disulfoton is usually applied to the soil at planting time or as a side dressing during cultivation in early post emergence. Broadcast foliar applications of granular formulations may be done in early post emergence, and may be applied by aircraft.

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Some information of the use summary above was extracted from the Qualitative Use Assessment Chapter of the Disulfoton Registration Standard.

OVERALL AND POLICY DISCUSSION

This section lists the minimum data requirements for the registration of a given pesticide, the toxicology data base for Disulfoton, and data gaps. It will identify and address also issues of toxicological concern and the need for tolerance reassessment.

A. Toxicology Data Requirements

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) designates the Environmental Protection Agency as the governmental body responsible for the safety of all pesticides used in the United States. In order for a pesticide to be Federally registered, it must be determined first that the pesticide does not pose unacceptable hazard to health or to the environment when used according to it's labeling restrictions.

The minimum data requirements(1) for the registration of a given pesticide are outlined in the 40 CFR Part 158 as follows:

acute oral toxicity
acute dermal toxicity
acute inhalation toxicity
primary dermal irritation
primary eye irritation
dermal sensitization
acute delayed neurotoxicitry

subchronic oral toxicity
subchronic dermal toxicity
subchronic inhalation toxicity

chronic feeding
oncogenicity
teratogenicity
reproduction
mutagenicity
metabolism



(1) It should be emphasized that particular use patterns and other factors may result in either the waiver of some data requirements or promote the need for additional data.

B. Toxicology Profile:

The Agency has reviewed and evaluated all the toxicology data available on technical Disulfoton and concluded the following:

1. Disulfoton is extremely toxic to mammals by all routes of administration, and classified, on the basis of acute toxicity, in the highest toxicity category. Generally females are much more susceptible to the toxic action of this chemical than males. Signs of acute intoxication, generally, are those typical of cholinergic poisoning. In a few cases, the acute toxicity of Disulfoton was extremely potentiated by other organophosphates.

2. Subchronic and chronic feeding studies on rats and dogs indicated also that cholinesterase is the primary target for the toxic action of Disulfoton. Higher mortality rate and fluctuation in blood chemistry parameters were observed at the high dose levels in the chronic studies in the rat. In the chronic and subchronic studies in dogs, a "no-observed effect level" is considered to be 1 ppm (0.025 mg/kg), and the "least effect level" is 2 ppm (0.05 mg/kg). On the otherhand a "no-observed effect level" could not be established for cholinesterase inhibition in the rat studies, since inhibition of cholinesterase was observed at the lowest dose level (1.0 ppm).

3. The chemical did not alter the spontaneous oncogenicity profile in CD-1 mice. The most frequently observed neoplastic lesion noted was malignant lymphoma, with the incidence being higher in the females. However, the incidences were similar among treated and control animals. There was a slight increase in the incidence of atrophy of the testes which was also coupled with a reduced sperm count in the mid- and high dose males; however, the increased incidence of these lesions did not attain a statistically significant level.

Although the chemical did not seem to alter the oncogenicity profile in the SPF Sprague-Dawley rats, the data as presented were inconclusive. Because of major deficiencies exist in the rat study, this report could not be considered a reliable assessment of the oncogenic potential of Disulfoton.

4. The chemical did not produce teratogenic effects in the rat. The treatment caused incomplete ossification of the parietals and sternebrae. A "no-observed effect level" for this fetotoxic effect was considered to be 0.3 mg/kg/day, and the "least effect level" was considered to be 1.0 mg/kg.

   In the rabbit, although the chemical did not seem to induce any teratogenic effect, major deficiences in the report precluded an adequate assessment of the teratogenic potential of this chemical.

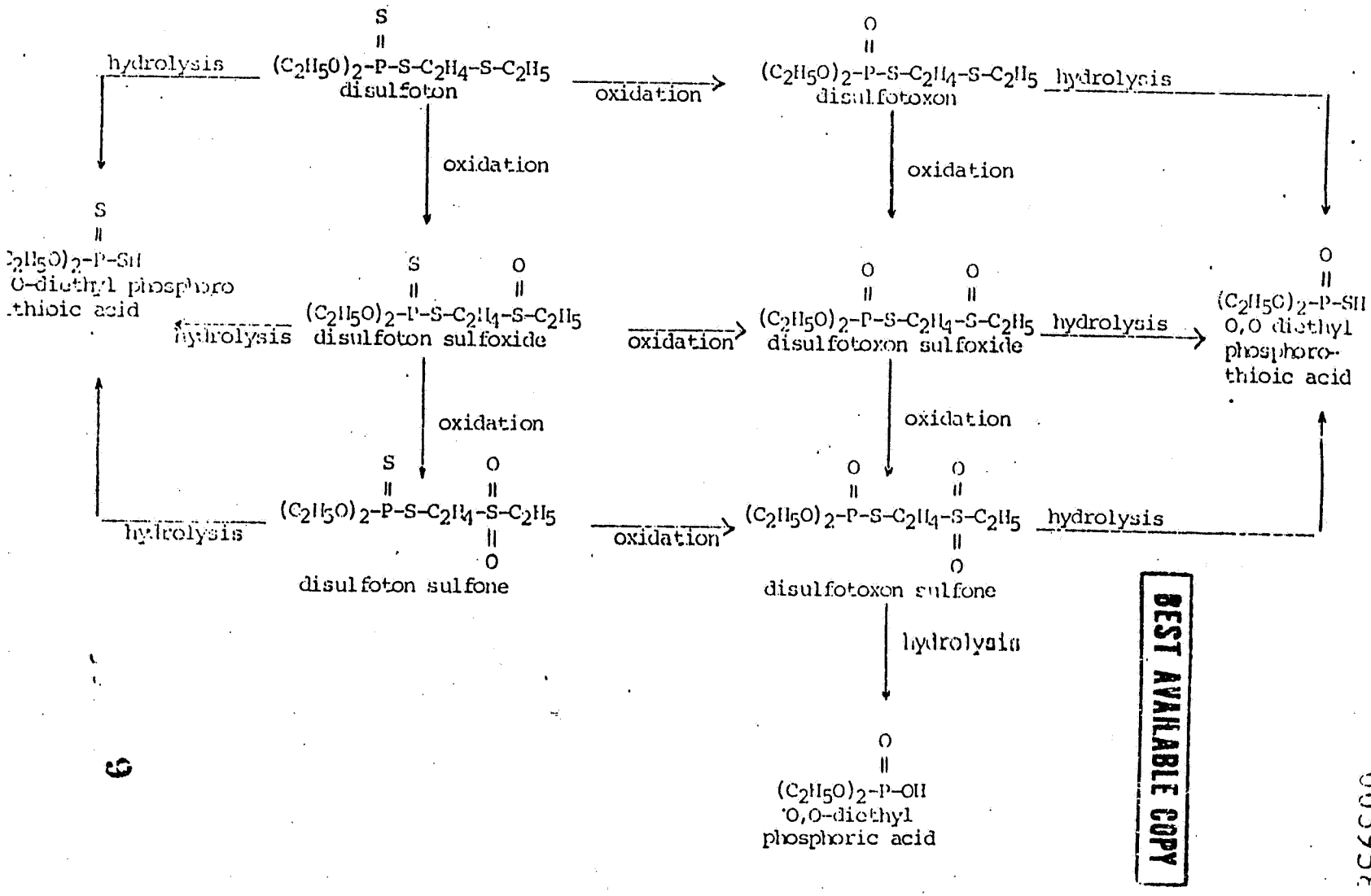
5. Apart from a reduction in litter size, lower viability index and lower pregnancy rate at high dietary level (10 ppm, or 0.5 mg/kg/day), the chemical did not seem to have major effects on reproductive parameters. However, the treatment caused juvenile hyperplasia in the testes of males in the high dose group (10 ppm). Since no histopathological data were available on other dosage levels, it was concluded that juvenile hyperplasia could be a treatment-related effect, and a "no-observed effect level" could not be established. A similar effect on the testes was observed in the chronic feeding study in mice and was manifested as atrophy. The report is generally judged as inconclusive and could not be used as an adequate assessment for the effect of disulfoton on reproduction.

6. Contradicting reports are available on the mutagenic potential of Disulfoton. The chemical was reported to be mutagenic in the reverse mutation assay in S. typhimurium, and E. coli. Disulfoton was reported to induce unscheduled DNA synthesis in human fibroblast cells. In all cases the positive mutagenic response was obtained without metabolic activation. However, Demeton, a registered pesticide and a primary metabolite of Disulfoton, was reported to be mutagenic in other studies. On the otherhand, Disulfoton was not mutagenic in several other in vitro microbial and in vivo mammalian test. It is therefore concluded that the mutagenic potential of Disulfoton is not adequately defined, and further testing is required.

7. The metabolism of Disulfoton, like other phosphorodithioates, may involve sequential oxidation steps that enhance the anti-cholinesterase properties, and hydrolytic or catalytic cleavage that render the chemical inactive (Fig. 1). The oxidation products may include the O-analog of Disulfoton and the sulfoxide and sulfon derivatives of both Disulfoton and its O-analog. The hydrolysis products may include: 0,0-diethyl-phosphoric; 0,0-diethyl-phosphorothioic; and 0,0-diethyl-phosphorodithioic acids.

FIGURE 1

SUGGESTED METABOLIC PATHWAY OF DISULFOTON



Administration of a single oral dose of radiolabeled Disulfoton to rats resulted in recovery of 96-99% of the administered dose as excretion products; 81.6 in urine, 7.0% in feces, and 9.2% as expired carbon dioxide. Excretory pathways were similar for males and females, but the rate of excretion was slower for females.

The major urinary metabolites were Diethylphosphate, and diethylphosphorodithioate. Small amounts of the oxidative metabolites were also recovered in urine and included the oxygen analog sulfoxide, oxygen analog sulfon, and Disulfoton sulfoxide. The ratio of the diethylphosphate to diethylphosphorothioate was higher for females than for males.

Tissues and blood levels peaked at about six hours after administration. Females accumulated a larger percent of the dose in the liver than males. Organosoluble metabolites in liver were identified as Disulfoton sulfoxide and the oxygen analog sulfoxide and sulfon. The higher toxicity of Disulfoton to females may be attributed to their lower capability for hydrolytic detoxication of the molecule.

8. Domestic Animal Safety: With a highly toxic chemical such as Disulfoton, the risk of accidental acute poisoning of domestic animals can not be precluded. Cases of acute poisoning of pets and livestock are reported in the literature.

Poisoning of pets, in most cases, may be the result of access to treated areas, stored or discarded pesticide containers, or contaminated food. Most cases of livestock poisoning occurred as a result of grazing in fields too soon after the application of the pesticide, as a result of drift from the application of the pesticide to adjacent areas, or drinking from runoff irrigation water from disulfoton treated areas.

Reports available on pesticide poisoning of domestic animals indicate that the majority of these cases are a result of human error or negligence. It is therefore recommended that a clause be added to the label of all Disulfoton-containing products to alarm consumers and to ensure the safety of domestic animals.

In general, evaluation of the toxicology data available on the technical Disulfoton indicated that they fall in one of the following categories:

1. Studies considered satisfactory and adequate to fulfill toxicology requirements; these include acute oral toxicity, acute dermal toxicity, potentiation of acute toxicity, teratogenicity in the rat, two-year feeding in the dog, and oncogenicity in mice.

2. Studies considered to be lacking or unsatisfactory in fulfilling toxicology data requirements*; these include acute and delayed neurotoxicity, subacute inhalation toxicity, subchronic dermal toxicity, chronic feeding and oncogenicity in the rat, teratogenicity in a second species (other than the rat), multigeneration reproduction, and mutagenicity, and metabolism.

*Classification of a study in this category does not necessarily mean that this study is considered as a data gap, or imply that it will be required. See data gaps and explanation on the following page, and footnote for the toxicology data requirements on the first page.

C. Data Gaps:

Based on the toxicology data requirements the following studies are considered to constitute data gaps for being incomplete (classified as supplementary), partially fulfilled or missing altogether (no data submitted), from the data base:

| Required Study | Current Status |
|---|---|
| Acute inhalation toxicity | Supplementary |
| Primary eye irritation | No data(1) |
| Primary dermal irritation | No data(1) |
| Dermal sensitization | No data(2) |
| Acute delayed neurotoxicity | Supplementary |
| Potentiation of acute toxicity | Partially fulfilled(3) |
| Subchronic feeding toxicity | Supplementary(4) |
| Subchronic dermal toxicity | Supplementary(5) |
| Subacute inhalation toxicity | Supplementary(6) |
| Chronic feeding | Partially fulfilled(7) |
| Oncogenicity | Partially fulfilled(7) |
| Teratogenicity | Partially fulfilled(9) |
| Reproduction | Supplementary |
| Mutagenicity | Partially fulfilled(9) |
| Metabolism | Supplementary |
| Other studies deemed necessary, but lacking | No data(10) |

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1. These requirements are waived. The chemical is too toxic to allow the use of the appropriate dose levels required for adequate testing.

2. Testing is not required. In addition to the extreme acute toxicity, the chemical structure of disulfoton does not indicate a possible dermal sensitization potential.

3. Testing is required. See explanation under F-3.

4. Retesting is not necessary. The existing studies indicated that cholinesterase is the primary target for the toxic action of Disulfoton. A "no-observed effect level" has been established for this parameter in a long-term feeding study in the dog.

5. This requirement is contingent upon results of worker exposure analysis.

(Footnotes continue on next page)

(Footnotes continues)

6. This requirement is contingent upon worker exposure analysis and product integrity studies on the granular formulations.

7. Testing is required in the rat.

8. Testing is required in another species (other than the rat).

9. Mammalian in vitro and in vivo studies are required.

10. Subchronic feeding studies using the oxidation metabolites. These studies are deemed necessary for tolerance reassessment purposes.

D. Tolerances:

Tolerances ranging from 0.01 to 0.75 ppm have been established for the combined residues of Disulfoton and its cholinesterase metabolites, calculated as demeton (40 CFR 180.183) in or on the following raw agricultural commodities:

1. Published Tolerances

| CROP | TOLERANCE(1) | FOOD FACTOR(2) | mg/kg (1.5 kg die |
|---|---|---|---|
| Barley | 0.750 | 0.03 | 0.00034 |
| Beans, dry edible | 0.750 | 0.31 | 0.00349 |
| Beans, lima | 0.750 | 0.19 | 0.00214 |
| Beans, snap | 0.750 | 0.98 | 0.01104 |
| Broccoli | 0.750 | 0.10 | 0.00115 |
| Brussel Sprouts | 0.750 | 0.03 | 0.00034 |
| Cabbage, sauerkraut | 0.750 | 0.74 | 0.00828 |
| Cauliflower | 0.750 | 0.07 | 0.00080 |
| Cottonseed (oil) | 0.750 | 0.15 | 0.00169 |
| Lettuce | 0.750 | 1.31 | 0.01472 |
| Oats | 0.750 | 0.36 | 0.00402 |
| Peanuts | 0.750 | 0.36 | 0.00402 |
| Peas | 0.750 | 0.69 | 0.00782 |
| Pecans | 0.750 | 0.03 | 0.00034 |
| Pineapple | 0.750 | 0.30 | 0.00333 |
| Potatoes | 0.750 | 5.43 | 0.06105 |
| Rice | 0.750 | 0.55 | 0.00621 |
| Sorghum | 0.750 | 0.03 | 0.00034 |
| Spinach | 0.750 | 0.05 | 0.00057 |
| Tomatoes | 0.750 | 2.87 | 0.03234 |
| Hops | 0.500 | 0.03 | 0.00023 |
| Sugar, cane & beet | 0.500 | 3.64 | 0.02729 |
| Coffee | 0.300 | 0.75 | 0.00336 |
| Corn, all types | 0.300 | 2.51 | 0.01130 |
| Wheat | 0.300 | 10.36 | 0.04663 |
| Peppers | 0.100 | 0.12 | 0.00018 |
| Soybeans (oil) | 0.100 | 0.92 | 0.00138 |

2. Unpublished, Approved

| CROP | TOLERANCE | FOOD FACTOR | mg/day/1.5 kg |
|---|---|---|---|
| Meat, red | 0.050 | 10.81 | 0.00811 |
| Milk & Dairy Product | 0.010 | 28.62 | 0.00429 |

(1) Parts per million

(2) Percent of food intake

The provisional acceptable daily intake (PADI) for man was established using a no-observed effect level (NOEL) of 1.0 ppm for cholinesterase inhibition generated in a chronic feeding study in the dog using a customary safety factor of 10 fold. The PADI was calculated to be 0.0025 mg/kg/day, and the maximum permissable intake (MPI) for a 60 kg person was calculated to be 0.1500 mg/day.

The theoritical maximum residue contribution (TMRC) to the human diet from the existing tolerances (published, 40 CFR 180.183) is 0.2544 mg/day/1.5 kg of the diet or 169.59% of the ADI. The TMRC has been increased to 0.2668 mg/day by the recent approval of tolerances for meat, milk, and dairy products (as per Pesticide Petition No. 7F1895). Granting of these tolerances, increased the utilized portion of the PADI to 177.8%.

These values are based on a NOEL generated as a result of direct exposure of dogs via the diet to the parent compound Disulfoton. However, plant residues were found to consist, almost entirely, of oxidation products that are far more toxic than the parent compound itself. The nature and magnitude of individual plant metabolites were not originally considered in the establishment of these tolerances.

E. Issues of Concern and Tolerance Reassessment:

Tolerance reassessment is considered an essential phase of the re-registration process of Disulfoton. There is a number of separate, but related issues that require to be addressed in order to define the problems associated with the tolerance reassessment. As it will be discussed later, part of the problem with the current tolerance of Disulfoton stems from the nature of this chemical, and the other part seems to be inherited in the tolerance system.

1. Nature of Plant Residues

Disulfoton is extremely toxic to mammalian systems on an acute basis, and apparently more toxic to females than to males. The acute oral $LD_{50}$ was determined to be 2.0 and 6.8 mg/kg in female and male rats respectively.

As is the case with almost all organophosphorus insecticides, acetylcholinesterase is the primary target for Disulfoton action. The cause of death is probably, the excessive stimulation of both parasympathetic and central nervous systems, and the consequent myoneural junction effect as a result of acetylcholine accumulation.

Although Disulfoton is a potent cholinesterase inhibitor, it can be further activated to more potent anticholinesterase metabolites via oxidative desulfuration and/or thioether oxidation. (Eto, 1974; Mann and Still, 1977; Fukuto and Metcalf, 1969; Nakatsugawa and Morelli, 1976; Kulkarni and Hodgson, 1980). The oxidation products include the O-analog of Disulfoton, and the sulfoxide and sulfon of both Disulfoton and its O-analog.

Evidence from in vitro studies indicate that the oxidation products are a few orders of magnitude more potent, as anticholinesterase, than the parent compound itself (March et al. 1957). The in vitro studies are also supported by in vivo data indicating that the acute oral $LD_{50}$ values for the oxidation products are far less than the $LD_{50}$ for the parent compound (Crawford and Anderson, 1974).

Plant metabolism studies, and residue data indicate that the residues consist almost entirely of oxidation products (Metcalf et al. 1959; Loeffler, 1970). The oxidation of Disulfoton to these more toxic metabolites is extremely rapid (Bull, 1965; 1965) and the oxidation products were shown to persist in the plants for a relatively long periods of time. There is no doubt that at the time of harvest the residue predominantly consists of oxidative metabolites, and the parent compound may not be even present in any detectable amount (Loeffler, 1970).

In view of the above, and considering the fact that the oxidation products, were previously discounted in the establishment of tolerance, it is concluded that, the actual toxic burden in the human diet as a result of existing tolerances is far greater than it appears to be, especially with a "provisional acceptable daily intake" already exceeded. It is therefore recommended that:

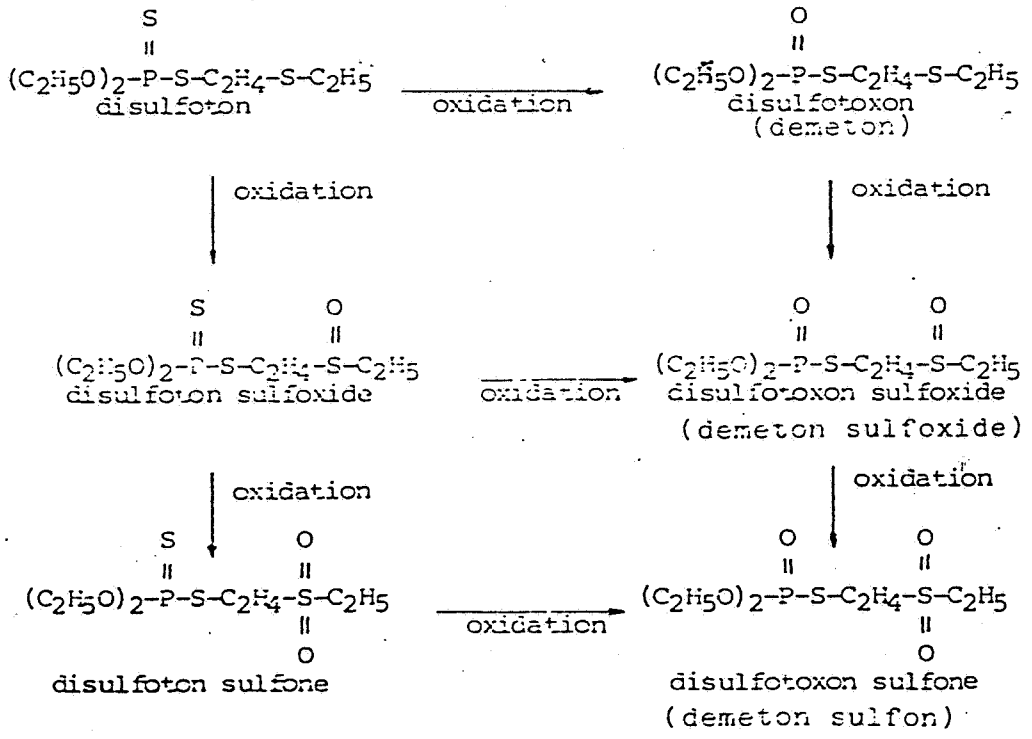
a. Any tolerance reassessment should be based on the actual constituents of the plant residues considering the nature and magnitude of individual metabolites, and the acceptable levels dictated by the most toxic metabolite of the group.

b. Existing tolerance levels should be reexamined to determine if they truly represent the actually occurring residues in/on food commodities or they are higher than the actual residue levels.

However, acceptable daily intake levels of Disulfoton oxidation metabolites remain to be established before a meaningful reassessment of tolerance becomes possible.

2. The Interrelation of Disulfoton and Other OP's:

The structural association of Disulfoton and other organophosphorus insecticides adds another dimension of complexity to the tolerance reassessment process. The oxidative desulfuration of the phosphorodithioate to phosphorothioate is well documented and is currently recognized as a major pathway in the biotransformation of this group of the organophosphorus pesticides. Disulfoton, a phosphorodithioate, can be metabolized in plant tissues, via this route, to the corresponding phosphorothioate. The latter is another pestiicde known as Demeton (or Systox) and registered for use on wide variety of raw agricultural commodities. In the same manner, the sulfoxide and sulfon of Disulfoton convert to the sulfoxide and sulfon of Demeton.



The actual theoritical maximum residue contribution of Disulfoton to human diet in reality is the combined resiudes of both chemicals. Since Demeton tolerances have been discounted in the calculation of the theoritical maximum residue contribution of Disulfoton to human diets, tnerefore, the actual toxic burden of Disulfoton residue to human diet as a result of existing tolerances, once again, is underestimated.

It is therefore necessary to account for the contribution of Demeton residues in the calculation of the overall theoritical maximum residue contribution of Disulfoton to human diet. This can be accomplished as follows: a) in case of crops for which registered uses exist for both Disulfoton and Demeton, the higher of the two tolerance levels should be used, and b) adding to the Disulfoton tolerance list, tolerances for Demeton residues on crops for which no registered uses for Disulfoton exist.

It should be emphasized that the compilation of the tolerances of the two chemicals is merely a quantitative accounting for tolerance, and should not be confused with the earlier recommendation to consider the actual plant residues in the reassessment of tolerances.

3. Infant Susceptibility and Existing Tolerance:

It should have been recognized by now, as a result of the preceding discussions that, the toxicity of the phosphorothioate or - dithioate insecticides is governed to various degrees by two competing mechanisms of biotransformation: a) oxidation that in most cases results in increasing anticholinesterase properties, and b) cleavage that renders the molecule inactive as a cholinesterase inhibitor. The overall toxicity is determined by the extent or the rate of each of these two contradicting processes in relation to the other.

Difficiency or lack of certain enzyme systmes in infants, and newly born is well documented. Thus, the balance of the activation and detoxication reactions might very well shift in favor of the activation, causing higher susceptibility of infants to this group of chemicals. Experimental evidence indicated that Disulfoton was twice as toxic to weanling than to adult rats (Brodeur and Du Bois, 1963) and also more toxic to newborn calves than to young cattle (McCarty et al. 1969).

The above discussion reveals a problem that might have been inherited into the current tolerance system, and triggers a question of vital importance. The question that naturally emerges as a result of the above discussion, is mainly concerned with the efficiency of the current tolerance system to provide adequate margins of safety for infants and newl born.

For instances, in the evaluation of milk tolerance, a calculation of infant daily intake was made for a 4 kg infant whose entire daily diet of 770 grams consists of 100% milk. At a tolerance level of 0.01 ppm, the infant intake of Disulfoton residues can be calculated as follows:

$$\text{Infant daily intake} = \frac{\text{food intake} \times \text{tolerance level}}{\text{body weight}}$$

$$= \frac{0.77 \text{ kg/day} \times 0.01 \text{ mg/kg}}{4 \text{ kg}} = 0.001925 \text{ mg/kg/day}$$

The permitted levels of pesticides in human diet is usually established based on a "no-observed effect level" in adult animals. The "acceptable daily intake" of Disulfoton residues has been set at 0.0025 mg/kg/day using (only) a 10 fold safety factor.

In this example, it is clear that the infant daily intake of Disulfoton residues is approaching, if not comparable, to the calculated "acceptable daily intake" for adults. Considering the extreme toxic nature of Disulfoton metabolites that might constitute most, if not all, the residues in milk, and in view of the higher susceptibility of infants, the tolerance level for milk (and possibly other baby food items) is considered unsafe, and does not provide an adequate margin of safety for infants and newly born.

F. Other Toxicological Issues and Human Safety:

1. Worker Exposure:

Disulfoton is normally used as aerial application or as soil dressing. Unless weather conditions are ideal for field application of pesticide, the chemical may drift creating a potential worker exposure problem. This is particularly true in case of aerial application of solutions. However in the case of the granular formulations, the problem may be different. Despite the use patterns and application practices of the granular formulations that may preclude possible exposure of workers, the possibility of inhalation and/or dermal exposure hazard might still exist. This may be particularly true if the granules would fracture during shipping and handling in a manner such that fine or respirable dust is generated.

For pesticides with low or even moderate toxicity, this issue may be of negligible importance, but with an extremely toxic chemical such as Disulfoton ($LD_{50}$; oral = 2.0 mg/kg, dermal 3.6 mg/kg), it should be a reason of major concern. Product integrity of granular formulations, and particle size distribution of the generated dust are issues remain to be investigated.

Should the outcome of this investigation indicate that such a situation is feasible, the Agency would then require either a) labeling which requires the use of dust masks and other protective measures by the occupationally exposed workers, and/or b) subacute inhalation exposure (21-day study in the rat), and subchronic dermal toxicity data, to assess the hazard of such exposure to human.

It is also recommended that an exposure profile be established for farm workers, and a reentry interval be determined to avoid or at least minimize unnecessary exposure to such a highly toxic chemical.

2. Ground Water Contamination:

Although foliar application uses are registered, disulfoton is mainly applied by incorporation into the soil where it is transferred as a systemic pesticide into the plant. It is reasonable therefore to assume that leaching may occur with possible contamination of ground water.

This would be more likely if enzymatic oxidation by soil microflora and/or non-enzymatic oxidation by other environmental factors occurred. Oxidation of Disulfoton would yeild metabolites or oxidation products which are not only more toxic but could be also more water soluble. Suett (MRID#00068098), and Shaw (MRID#00095669) reported that the applied Disulfoton converted almost entirely to the sulfoxide and sulfon shortly after application to the soil. The oxidation of Disulfoton was rapid and the oxidation products persisted in the soil for a relatively long period of time.

The environmental behavior of Disulfoton remains to be investigated before a risk assessment can be made, and safety criteria can be established.

3. Potentiation Interaction:

Disulfoton is one of the most acutely toxic pesticides to mammals by all routes of administration. The acute oral $LD_{50}$ in female rats was determined to be 2.0 mg/kg, and the acute dermal $LD_{50}$ was determined to be 3.6 mg/kg. The acute inhalation $LC_{50}$ in male mice was determined to be 0.05 mg/l. On the basis of acute toxicity the chemical is classified in the highest toxicity category.

The acute toxicity of Disulfoton was extremely potentiated when administered in combinations of equitoxic mixture with Phosalon, another phosphorodithioate insecticide. According to Scott and Beliles (1965), the acute toxicity of Disulfoton to female rats was potentiated over sixteen times. Since the acute toxicity of Disulfoton was not potentiated with other phosphate or phosphorodithioate insecticides (DuBois, 1957; Johnston, 1963, 1966), the potentiation interaction is most probably attributed to the heterocyclic moiety of the Phosalone molecule. However the mechanism responsible for this type of interaction is not yet defined.

For pesticides with a low or moderate acute toxicity, this issue may be of a little or no importance, but with an extremely toxic chemical as such, this situation should be a reason of concern. It is concluded that further investigation might still be required to clarify the issue of potentiation interaction, and to identify problems of this nature. The investigation of this problem should include the interaction of chemicals used on crops, in combination, as well as chemicals used in succession with Disulfoton.

4. Possible Product Mislabeling:

Customarily, pesticidal products with an acute oral $LD_{50}$ of 50 mg/kg or less, are classified in the highest toxicity category (class 1), and labeled as restricted use pesticides. Theoritically, formulations of Disulfoton containing 3.8% a.i. or more would have an acute oral $LD_{50}$ of 50 mg/kg or less, and should be labeled for restricted use only. Since some of the Disulfoton formulations are not labeled appropriately for restricted use, relabeling is therefore required unless data are submitted indicating otherwise.

# APPENDIX I

## SUMMARY OF TOXICOLOGY DATA
## "Toxicology One-Liner"

| Study/Lab/Study #/Date | Material | EPA Accession No. | Results | Tox Category | CORE Grade |
|---|---|---|---|---|---|
| MRID 00068347<br>Acute oral toxicity in rats and guinea pigs<br>Univerity of Chicago<br>Report No. 1732<br>Date - 11/20/57 | Di-Syston | | Acute oral $LD_{50}$;<br>Rat (female) = 2.6 mg/kg<br>Rat (male) = 12.5 mg/kg<br>Guinea pig (male) = 10.8 mg/kg | I | Supple-mentary |
| MRID 00071872<br>Acute oral toxicity in guinea pigs<br>Chemagro Research and Development<br>Report No. 39113<br>Date - 12/17/83 | Di-Syston 96% | | Acute oral $LD_{50}$<br>Male = 8.9 (5.6-14.1) mg/kg<br>Female = 12.7 (8.1-20.1) mg/kg | I | Supple mentary |
| MRID 05004291<br>Acute oral toxicity in weanling and adult male rats<br>Laboratory:<br>Report No.:<br>Date - 1963 | Di-Syston | | Acute oral $LD_{50}$ in rats;<br>Adult male = 9.4 (8.5-10.3) mg/kg<br>Weanling male = 5.4 (5.0-5.8) mg/kg | I | Supple-mentary |
| MRID 00071873<br>Acute oral toxicity of Disyston and its metabolites in rats<br>Laboratory:<br>Report No.: 39687<br>Date - 1974 | Di-Syston 96% and Di-Syston sulfoxide and sulfon | | Acute oral $LD_{50}$ in female rats;<br>Di-Syston = 2.0 (1.3-3.0) mg/kg<br>Sulfoxide = 1.7 (1.5-1.9) mg/kg<br>Sulfon = 1.24 ( ) mg/kg<br>Thiolsystox = 1.17 (0.96-1.4) mg/kg<br>Thiolsystox sulfoxide = 1.24 ( ) mg/kg<br>Thiolsystox sulfon = 1.10 (0.95-1.27) mg/kg | <br>I<br>I<br>I<br>I<br>I | Supple-mentary |

| Study/Lab/Study #/Date | Material | EPA Accession No. | Results | Tox Category | CORE Grade |
|---|---|---|---|---|---|
| MRID 000000000<br>Acute oral toxicity in rats, mice and dogs<br>Bayer AG, Institute of Toxicology<br>Report No.: 7602a<br>Date - 1/12/78 | Di-Syston technical 94.4% | 072293 | Acute oral $LD_{50}$;<br>Male rat = 6.2 (5.6-6.8) mg/kg<br>Female rat = 1.9 (1.6-2.2) mg/kg<br>Male mice = 7.0 (6.5-7.5) mg/kg<br>Female mice = 8.2 (7.5-9.0) mg/kg<br>Female dogs = approx. 5.0 mg/kg | I<br>I<br>I<br>I<br>I | Minimum<br><br>Minimum<br><br>Supple-mentary |
| MRID 000000000<br>Acute dermal toxicity in rats<br>Bayer AG, Institute of Toxicology<br>Report No.: 7062b<br>Date - 1/12/78 | S 276 Di-Syston 94.4% | | Acute dermal $LD_{50}$;<br>Male = 15.9 (14.8-17.0) mg/kg<br>Female = 3.6 (3.1-4.0) mg/kg | I | Minimum |
| MRID 00043213<br>Acute dermal toxicity in rats<br>University of Chicago<br>Report No.: 2063<br>Date - 1957 | Di-Syston technical<br>EC 2 lb/gal.<br>EC 8 lb/gal.<br>50% [redacted] powder,<br>50% [redacted] powder | | Acute dermal $LD_{50}$ in rats;<br>Technical = 20 mg/kg<br>EC 2 lb/gal = 84 mg/kg<br>EC 8 lb/gal = 20 mg/kg<br>50% [redacted] powder = 70 mg/kg<br>50% [redacted] powder = 60 mg/kg | I | Supple-mentary |
| MRID 00069349<br>Acute inhalation toxicity in rats and mice<br>University of Chicago<br>Report No.: 1802<br>Date - 11/20/57 | Di-Syston technical | | Acute inhalation $LC_{50}$;<br>Adult male rat = 0.2 mg/l<br>Adult male mice = 0.05 mg/l | I | Supple-mentary |



INERT INGREDIENT INFORMATION IS NOT INCLUDED

| Study/Lab/Study #/Date | Material | EPA Accession No. | Results | Tox Category | CORE Grade |
|---|---|---|---|---|---|
| MRID 00069347<br>Acute intraperitoneal toxicity in rats, mice and guinea pigs<br>University of Chicago<br>Report No.: 1732<br>Date - 11/20/57 | Di-Syston technical | | Acute intraperitoneal $LD_{50}$:<br>Male rats = 10.5 mg/kg<br>Female rats = 2.0 mg/kg<br>Male mice = 1.1 mg/kg<br>Female mice = 6.5 mg/kg<br>Male guinea pigs = 7.0 mg/kg | N/A | Supplemental information |
| MRID 00069531<br>Potentiation of acute toxicity in female rats<br>University of Chicago<br>Report No.: 1995<br>Date - 11/20/57 | Di-Syston plus other organic phosphates | | No potentiation interactions occurred with parathion, methylparathion, guthion, EPN, systox, malathion, trithion, and diazinon. | N/A | Supplementary |
| MRID 00075432<br>Potentiation of acute toxicity in rats<br>Woodard Research Laboratory<br>Report No.:<br>Date - 6/4/63 | Di-Syston 99%, plus technical Imidan | | Additive effect. | N/A | Supplementary |
| MRID 00011448<br>Potentiation of acute toxicity in rats<br>Woodard Research Laboratory<br>Report No.:<br>Date - 1966 | Di-Syston 99%, plus Methidathion | | Additive effect. | N/A | Supplementary |
| MRID 00006794<br>Potentiation of acute toxicity<br>Woodard Research Laboratory<br>Report No.:<br>Date - 1965 | Di-Syston 99%, plus Phosalone | | Significant potentiation interaction.<br>Acute oral $LD_{50}$ in rats = 3.61 mg/kg. The $LD_{50}$ was significantly reduced in the presence of phosalone. | N/A | Minimum |

| Study/Lab/Study #/Date | Material | Accession No. | Results | Tox Category | CORE Grade |
|---|---|---|---|---|---|
| MRID 00000000<br>Delayed neurotoxicity in hens<br>Mobay Chemical Corp.<br>Report No.: 365<br>Date - 3/7/83 | Di-Syston 97.8% | 072293 | The test chemical did not cause delayed neurotoxic effect. | N/A | Supplementary |
| MRID 00057265<br>Demyelination study on hens<br>Harris Laboratories, Inc.<br>Report No.: 15107<br>Date - 1/5/65 | Di-Syston | | The treatment did not cause demyelination in hens. | N/A | Supplementary |
| MRID 00069348<br>Subacute ip toxicity (60 days) to rats<br>University of Chicago<br>Report No.: 1967<br>Date - 11/20/57 | Di-Syston technical | | Dose scale: 0.0, 0.25, 0.5, 1.0, 1.2, and 1.5 mg/kg. The animals tolerated doses of up to 0.5 mg/kg with no mortality. A NOEL for brain and plasma cholinesterase could not be established. | N/A | Supplementary |
| MRID 00089399<br>Subchronic feeding (12 weeks) toxicity in dogs<br>University of Chicago<br>Report No.:<br>Date - 1958 | Di-Syston 25% WP | | Dose scale: 1.0, 2.0, and 10 ppm.<br>Plasma cholinesterase NOEL = 1.0 ppm, LEL = 2.0 ppm. | N/A | Supplementary |
| MRID 00089396<br>Subchronic feeding (16 weeks) toxicity in the rat<br>University of Chicago<br>Report No.:<br>Date - 1958 | Di-Syston 25% WP | | Dose scale: 0.0, 1.0, 2.0, 5.0, and 10 ppm.<br>A NOEL for brain cholinesterase could not be established. Female brain cholinesterase was inhibited 11% at 1.0 ppm. | N/A | Supplementary |

| Study/Lab/Study #/Date | Material | EPA Accession No. | Results | Tox Category | CORE Grade |
|---|---|---|---|---|---|
| MRID 00087935<br>Subacute inhalation toxicity in female rats<br>University of Chicago<br>Report No.:<br>Date - 1971 | Di-Syston | | Cholinesterase inhibition<br>NOEL = 0.00075 mg/m$^3$ (HDT) | N/A | Supplementary |
| MRID 00073344<br>Subacute dermal toxicity to female rats<br>Laboratory:<br>Report No.: 21923<br>Date - 1968 | Di-Syston technical, Di-Syston sulfoxide sulfon | | Dose scale: 0.1 mg/kg daily for five days.<br>Serum cholinesterase was inhibited approx. 50% in all cases after 5 days. | N/A | Supplementary |
| MRID 00000000<br>Subacute inhalation rats<br>Bayer Institute of Toxicology<br>Report No.: 9065<br>Date - 4/1/80 | S 276<br>Di-Syston<br>94.4% | 072293 | Plasma, RBC, and brain cholinesterase inhibition at all dose levels including the lowest dose (0.02 mg/m$^3$). | N/A | Supplementary |
| MRID 00069966<br>Two-year feeding/oncogenicity study in the rat<br>Sandoze Ltd.<br>Report No.: 47069<br>Date - 1975 | Di-Syston technical<br>95.7% | 099417 | Dose scale: 0.0, 0.5, 5.0, 1.0, 2.0.<br>NOEL for brain cholinesterase could not be established for females.<br>The oncogenicity phase of this report was inconclusive. | N/A | Supplementary |
| MRID 00073348<br>Two-year feeding in the dog<br>Bayer AG<br>Report No.: 45287<br>Date - 1975 | Di-Syston technical<br>95.7% | 099417 | Plasma and RBC cholinesterase NOEL is 1 ppm, and LEL is 2 ppm. | N/A | Minimum |

| Study/Lab/Study #/Date | Material | No. | Results | Category | Grade |
|---|---|---|---|---|---|
| MRID 000000000<br>Oncogenicity study on Mice<br>Mobay Chemical Corporation<br>Report No.: 80-271-04<br>Date - 8/10/83 | Disulfoton technical 98.2% | 072293 | Not oncogenic. | N/A | Minimum |
| MRID 00091104<br>Three-generation reproduction in the rat<br>Harris Laboratories, Inc.<br>Report No.:<br>Date - 5/5/66 | Di-Syston technical 98.5% | | Dose scale: 0.0, 2.0, 5.0, and 10 ppm.<br>Dietary level of 10 ppm caused reduction in litter size, pregnancy rate, viability index, in addition, to juvenile hyperplasia in males.<br>The report was inconclusive, a NOEL could not be established. | N/A | Supplementary |
| MRID 000000000<br>Teratology in the rabbit<br>Life Science Research<br>Report No.: 2351<br>Date - 12/22/82 | S 276 Di-Syston 97.3% | 072293 | Not teratogenic. | N/A | Supplementary |
| MRID 000000000<br>Teratology in the rat<br>Mobay Chemical Corp.<br>Report No.:<br>Date - 5/13/83 | Di-Syston 98.2% | 072293 | Not teratogenic<br>Fetotoxicity NOEL 0.3 mg/kg, LEL 1.0 mg/kg. | N/A | Minimum |
| Mutagenicity: | | | | | |
| MRID 000000000<br>Micronucleus test in mouse<br>Bayer Institute of Toxicology<br>Report No.: 10451<br>Date - 12/23/81 | S 276 Di-Syston 50% | 250895<br>072293 | Not mutagenic. | N/A | Unacceptable |

| Study/Lab/Study #/Date | Material | EPA Accession No. | Results | Tox Category | CORE Grade |
|---|---|---|---|---|---|
| MRID 000000000<br>Sister chromatid exchange<br>Roswell Park Memorial Institute<br>Report No.:<br>Date - 1981 | Di-Syston 98.6% | 072293 | Not mutagenic without metabolic activation. The test was not performed in the presence of metabolic activation system. | N/A | Unacceptable |
| MRID 000000000<br>Dominant lethal in male mouse<br>Bayer Institute of Toxicology<br>Report No.: 9440<br>Date - 12/23/80 | S 276<br>Di-Syston 94.9% | 072293 | Not mutagenic. | N/A | Unacceptable |
| MRID 00000000<br>Rec-assay in B. subtilis<br>Nitokumo Agr. Chem. Institute<br>Report No.: AC86190<br>Date - 6/30/76 | Di-Syston 94.1% | 072293 | Not mutagenic. | N/A | Unacceptable |
| MRID 000000000<br>Reverse mutation in S. typhimurium<br>Nitokumo Agr. Chem. Institute<br>Report No. AC86190<br>Date - 6/30/76 | Di-Syston 94.1% | 072293 | Not mutagenic. | N/A | Unacceptable |
| MRID 000000000<br>Reverse mutation in S. cerevisia<br>Litton Bionetics, Inc.<br>Report No.: 2087 | Di-Syston | 072293 | Not mutagenic. | N/A | Unacceptable |

| Study/Lab/Study #/Date | Material | EPA Accession No. | Results | Tox Category | CORE Grade |
|---|---|---|---|---|---|
| MRID 000000000<br>Effect on growth of mammalian cells<br>Roswell Park Memorial Institute<br>Report No.: AC 86185<br>Date - 1973 | Di-Syston 96.8% | 072293 | Cytotoxic to human hematopoietic cells. | N/A | Unacceptable |
| MRID 000000000<br>Reverse mutation in S. typhimurium<br>Reverse mutation in E. coli<br>Monash University<br>Report No.:<br>Date - 1975 | Di-Syston 99.3% | 072293 | Mutagenic<br>Mutagenic | N/A | Acceptable |
| MRID 000000000<br>Dominant lethal study in male mouse<br>Bayer AG, Institute of Toxicology<br>Report No.: 9440<br>Date - 1980 | Di-Syston technical 94.9% | | The chemical did not induce mutagenic response. | N/A | Unacceptable |
| MRID 00028625<br>In vitro microbial mutagenicity studies<br>SRI International<br>Report No.: EPA-600/1-79-041<br>Date - 1979 | | | | | |
| 1) Reverse mutation in S. typhimurium | | | The chemical did not induce mutagenic response. | | Unacceptable |
| 2) Reverse mutation in E. coli WP2 | | | The chemical did not induce mutagenic response. | | Unacceptable |
| 3) Recombination assay in S. cerevisia D3 | | | The test material did not induce mutagenic response. | | Unacceptable |

| Study/Lab/Study #/Date | Material | EPA Accession No. | Results | Tox Category | CORE Grade |
|---|---|---|---|---|---|
| 4) DNA repair in E. coli | | | The chemical did not induce mutagenic response. | | Unacceptable |
| 5) DNA repair in B. subtilis H17/M45 | | | The chemical did not induce mutagenic response. | | Unacceptable |
| MRID 00028625<br>Unscheduled DNA synthesis in human fibroblast<br>SRI International<br>Report No. EPA-600/1-79-041<br>Date - 1979 | Di-Syston | | The chemical was positive in the absence of the metabolic activation system. | N/A | Acceptable |
| MRID 00013487<br>Acute oral toxicity to farm livestock | Di-Syston EC 65.7% | | NOEL for a single oral dose;<br>Newborn calves = 0.25 mg/kg<br>Yearling cattle = 0.5 mg/kg<br>Sheep = 1.0 mg/kg<br>Goats = 1.0 mg/kg | N/A | Supplemental Data |
| MRID 000000000<br>Metabolism and excretion of Di-Syston by rats<br>Mobay Chemical Corporation<br>Report No.: 44261<br>Date - 5/6/75 | $^{14}C$-labeled Di-Syston | | The metabolism of Disulfoton may involve sequential oxidation and hydrolytic cleavage. 96-99% of the administered dose was recovered as excretion product; 81.6% in the urine, 7% in feces, 9% as expired $CO_2$. Urine metabolites included Disulfoton sulfoxide, the sulfoxide and sulfon of the O-analog, diethylphosphate, and dietylphosphorothioate. | N/A | Supplementary |

| Study/Lab/Study #/Date | Material | EPA Accession No. | Results | Tox Category | CORE Grade |
|---|---|---|---|---|---|
| MRID 00083251<br>Metabolism of Disulfoton in mice<br>University of California<br>Report No.:<br>Date - 1957 | $^{32}P$-labeled Di-Syston | | The metabolism of Di-Syston involves sequential oxidation of the thioether sulfur and/or oxidative desulfuration, in addition to hydrolytic cleavage.<br><br>The oxidation results in increased anticholinesterase properties while the hydrolytic cleavage causes the loss the anticholinesterase properties.<br><br>Most of the [illegible] was recovered as hydrolysis products in the urine. Very small amount was recovered the the feces.<br><br>The in vitro metabolism using liver slices resulted in the formation of the sulfoxide and sulfon of Disulfoton, and sulfoxide and sulfon of the O-analog of Disulfoton. | N/A | Supplementary |

# APPENDIX II

## GENERIC DATA REQUIREMENTS
## "Technical Disulfotan"

TABLE A
GENERIC DATA REQUIREMENTS FOR DISULFOTON

| Data Requirement | Composition[1/] | Use Patterns[2/] | Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially | Bibliography Citation | Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/] |
|---|---|---|---|---|---|
| §158.135 Toxicology | | | | | |
| ACUTE TESTING: | | | | | |
| 81-1 - Oral $LD_{50}$ - Rat | TGAI | A,B,C,D,E,F, G,H | Yes | Mihail, F. (1978) | No |
| 81-2 - Dermal $LD_{50}$ | TGAI | A,B,C,D,E,F, G,H | Yes | Mihial, F. (1978) | No |
| 81-3 - Innalation $LC_{50}$ - Rat | TGAI | A,B,C,D,E,F, G,H | No | | Yes |
| 81-7 - Acute Delayed Neurotoxicity - Hen | TGAI | A,B,C,D,E,F, G,H | No | | Yes |
| SUBCHRONIC TESTING: | | | | | |
| 82-1 - 90-Day Feeding - Rodent, Non-rodent | TGAI | A, C, E | No | | Yes(4) |
| 82-2 - 21-Day Dermal | TGAI | A, E, F, G | No | | Yes(5) |
| 82-3 - 90-Day Dermal | TGAI | | No | | No |
| 82-4 - 90-Day Inhalation - Rat | TGAI | | No | | Yes(6) |
| 82-5 - 90-Day Neuro-toxicity - Hen/ Mammal | TGAI | | No | | Yes(7) |

1/Composition: TGAI - Technical grade of the active ingredient.
2/The use patterns are coded as follows: A-Terrestrial, Food Crop; B-Terrestrial, Non-Food; C-Aquatic, Food Crop; D-Aquatic, Non-Food, E-Greenhouse, Food Crop; F-Greenhouse, Non-Food; G-Forestry; H-Domestic Outdoor; I-Indoor.
3/Data must be submitted no later than _____.

(Footnotes continues on next page)

(Footnotes continued)

4/Using the oxidation metabolites of Disulfoton.

5/Only if worker exposure analysis indicates potential hazard of repeated exposure.

6/Only if worker exposure analysis indicates potential hazard of repeated exposure, and if product integrity studies indicate that respirable dust is generated during shipping and handling (see data gaps).

7/Contingent upon the outcome of the acute delayed neurotoxicity study.

GENERIC DATA REQUIREMENTS FOR DISULFOTON

| Data Requirement | Composition[1] | Use Patterns[2] | Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially | Bibliography Citation | Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3] |
|---|---|---|---|---|---|
| §158.135 Toxicology (continued) | | | | | |
| CHRONIC TESTING: | | | | | |
| 83-1 - Chronic Toxicity - 2 species: Rodent and Non-rodent | TGAI | A, C, E | Partially | Hoffmann et al. (1975) | Yes(4) |
| 83-2 - Oncogenicity Study - 2 species: Rat and Mouse preferred | TGAI | A, C, E | Partially | Hays (1983) | Yes(5) |
| 83-3 - Teratogenicity - 2 species | TGAI | A,B,C,,E,F, G,H, I | Partially | Lamb et al. (1983) | Yes(6) |
| 83-4 - Reproduction, 2-generation | TGAI | A,B,C,D,E,F, G | No | | Yes |
| MUTAGENICITY TESTING: | | | | | |
| 84-2 - Gene Mutation | TGAI | A,B,C,E,F,G, I | Partially | Hanna and Dyer (1975) | Yes(7) |
| 84-2 - Chromosomal Aberration | TGAI | | No | | Yes(8) |
| 84-2 - Other Mechanisms of Mutagenicity | TGAI | | Partially | Simmon (1979) | Yes |

1/Composition: TGAI - Technical grade of the active ingredient.
2/The use patterns are coded as follows: A-Terrestrial, Food Crop; B-Terrestrial, Non-Food; C-Aquatic, Food Crop; D-Aquatic, Non-Food, E-Greenhouse, Food Crop; F-Greenhouse, Non-Food; G-Forestry; H-Domestic Outdoor; I-Indoor.
3/Data must be submitted no later than _____.

(Footnotes continues on next page)

(Footnotes continued)

4/Testing required in the rat.

5/Testing required in the rat.

6/Testing required in another species (other than the rat).

7/*In vitro* mammalian cell; L5178 (TK), CHO (HGPRT), or V79 (HGPRT)

8/Sister chromatid exchange in CHO cells or human lymphocytes and dominant lethal.

9/Contingent upon the outcome of 7 and 8 above.

TABLE A
GENERIC DATA REQUIREMENTS FOR DISULFOTON

| Data Requirement | Composition[1/] | Use Patterns[2/] | Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially | Bibliography Citation | Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/] |
|---|---|---|---|---|---|
| §158.135 Toxicology | | | | | |
| SPECIAL TESTING: | | | | | |
| 85-1 - General Metabolism | PAI or PAIRA | A, C, F, I | Yes | March et al. (1957) Pull & Fredrickson (1975) | Yes |
| 85-2 - Domestic Animal Safety | Choice | A, B | Yes | McCarty et al. (1969( | No |

[1/]Composition: PAI = Pure active ingredient; PAIRA = Pure active Ingredient, radiolabelled; Choice = Choice of several test substances determined on a case-by-case basis.

[2/]The use patterns are coded as follows: A-Terrestrial, Food Crop; B-Terrestrial, Non-Food; C-Aquatic, Food Crop; D-Aquatic, Non-Food; E-Greenhouse, Food Crop; F-Greenhouse, Non-Food; G-Forestry; H-Domestic Outdoors; I-Indoors.

[3/]Data must be submitted no later than _____.

# APPENDIX III

## DATA EVALUATION RECORDS
## "Confidential Business Information"

# Acute Oral Toxicity in Rats and Guinea Pigs

Fiche/Master ID 00068347

Bombinski, T.J.; DuBois, K.P. (1957) The Acute Mammalian Toxicity and Pharmacological Actions of Di-Syston: Report No. 1732. (Unpublished study received Nov. 20, 1957 under 3125-58; prepared by Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL: 100152-B)

Test Chemical:

Technical Di-Syston with unspecified purity.

Experimental Protocol:

The test material was dissolved in 10% ethanol in propylene glycol and administered orally to a total of 30 male and 25 female Sprague-Dawley rats, and 27 male guinea pigs. The animals were observed for a period of 10 days for toxicity signs and mortality. The $LD_{50}$ was calculated using the logarithm-probability method.

Results:

The symptoms of acute intoxication were manifested as excitability, salivation, lacrimation, defecation, and muscular fasciculations followed by convulsive seizures, prostration and cessation of respiration. The time of onset and duration of symptoms were dependant on the dose. Death usually occurred within 48 hours or longer depending on the dose.

The $LD_{50}$ values were determined to be 12.5, 2.6 and 10.8 mg/kg for male and female rats and male guinea pigs respectively.

Conclusions:

On the basis of the acute oral toxicity, the test chemical is assigned to Toxicity Category I.

Core Classification:

Core Supplementary data. Experimental details and raw data were not provided to ascertain the accuracy of the $LD_{50}$ values reported.

Acute Oral Toxicity in Rats, Mice and Dogs

Fiche Master ID 000000900

Mihail, F. (1978). S 276 (Disyston Active Ingredient) acute toxicity studies. Report No. 7602a prepared by Bayer AG, Institut Fur Toxikologie for Mobay Chemical Corporation. June 12, 1978.

Test Chemical:

Di-Syston technical 94.4%.

Experimental Protocol:

1. This study used 120 male and 105 female Wistar rats with body weights of 170-230 g, 90 male and 90 femael NMRI mice weighing 18-22 g, and 6 female beagle dogs weighing 9.5 to 15.0 kg. Rats and mice, caged separately, were housed in Makrolon® cages (number per cage not specified) and provided food and water ad libitum. The dogs were individually housed in concrete kennels and provided Altromin H Diet and water ad libitum.

2. All animals were fasted for 16 hours before administration of the test material. Groups of 15 male rats each, were dosed by gavage with 1.0, 4.0, 4.5, 5.0, 6.0, 7.5, 9.0, or 10.0 mg/kg of the test material emulsified in Cremophor EL and distilled water; the dose volume was 10 ml/kg body weight. Groups of 15 female rats each, were dosed with 0.5, 1.0, 1.25, 1.5, 2.0, 2.5, or 5.0 mg/kg.

3. Each group of 15 male mice received the test material at dose levels of 2.5, 5.0, 6.0, 7.0, 8.0, or 10.0 mg/kg while each group of 15 female mice received test material doses of 2.5, 5.0, 6.5, 7.5, 10.0, or 11.0 mg/kg; the dose volume was 10 ml/kg body weight.

4. One dog identified as a female received 1.0 mg/kg, two dogs received 2.0 mg/kg, two received 5.0 mg/kg, and one received 10 mg/kg. Doses were administered at a volume of 2.5 ml/kg of body weight.

5. Observations for toxic signs were made at unspecified intervals for 14 days after administration of test material. Necropsy was performed on rats and dogs that died during the study.

RESULTS:

Toxic signs ("impairment of general health condition", muscle twitching, clonic cramps, breathing disorders, and salivation) were observed in all rats and mice at all dose levels except the lowest dose. The onset of these signs was within 1-2 hours of dosing for the lower doses and within 30 minutes at the nigher doses.

In dogs, toxic signs included "impairment of general health condition", muscle cramps, breathing disorders, vomiting, and diarrhea. In the 2.0 and 5.0 mg/kg groups, it was reported that the "test compound (was) vomited".

The signs of toxicity, especially, "impairment of the general health condition" and "breathing disorders", were observed in an unspecified number of animals and dose groups for 8 days after dosing. The number of deaths and the time of death for animals at the various dose levels are shown in Table 1.

At necropsy "mild pulmonary edemas" were observed in rats that died during the study. No gross pathological alterations of the inner organs were seen in rats at sacrifice; no comment was made concerning the results of gross necropsy of the mice. "Massive hemorrhagic pulmonary edemas" were observed in the 2 dogs that died during the study; one dog had a spleen that was "spotted and had a rough surface."

DISCUSSION:

As a determination of acute oral toxicity, this study was adequately conducted and reported; however, several minor limitations were noted in the test procedures and data reporting. The age of the animals used was not reported although the initial body weight ranges were given. There was no indication that the individual body weights were recorded during the observation period. Observations made during necropsy for the mice were not presented.

Only female dogs were used and only one or two dogs were used at each dose level. In addition, the dogs at the two mid-dose levels vomited, thus reducing their actual dose. Therefore, only supplementary data are available for dogs.



[redacted]

CONCLUSIONS:

Under the conditions of this study, the oral $LD_{50}$ of the test material was as follows: male rats - 6.2 mg/kg (5.6 - 6.8); female rats - 1.9 mg/kg (1.6 - 2.2); male mice 7.0 mg/kg (6.5 - 7.5), and female mice - 8.2 mg/kg (7.5 - 9.0).

CORE CLASSIFICATION: Minimum Data - for rats and mice.
Supplementary - for dogs.

TOXICITY CATEGORY: I.

TABLE 1. Acute Oral Toxicity Results

003058

| Dose in mg/kg | Toxicological Results** | Occurrence of death after | $LD_{50}$ (95 percent confidence interval) mg/kg |
|---|---|---|---|
| | | Male rats | |
| 1.0* | 0/0/15 | - | |
| 4.0 | 0/15/15 | - | |
| 4.5 | 1/15/15 | 4d | |
| 5.0 | 5/15/15 | 2-3h | 6.2 |
| 6.0 | 9/15/15 | 1-4h | (5.7 - 6.8) |
| 7.5 | 11/15/15 | 2h-1d | s = 1.2 |
| 9.0 | 13/15/15 | 1-2h | |
| 10.0 | 15/15/15 | 1h | |
| | | Females rats | |
| 0.5* | 0/0/15 | - | |
| 1.0 | 0/15/15 | - | |
| 1.25 | 1/15/15 | 4d | 1.9 |
| 1.5 | 1/15/15 | 3d | (1.6 - 2.2) |
| 2.0 | 10/15/15 | 1-4h | s = 1.2 |
| 2.5 | 13/15/15 | 2h-2d | |
| 5.0 | 15/15/15 | 1h | |
| | | Male mice | |
| 2.5* | 0/0/15 | - | |
| 5.0 | 0/15/15 | - | |
| 6.0 | 3/15/15 | 1h-1d | 7.0 |
| 7.0 | 7/15/15 | 1h-3d | (6.5- 7.5) |
| 8.0 | 12/15/15 | 1-2h | s = 1.2 |
| 10.0 | 15/15/15 | 1h-6d | |
| | | Female mice | |
| 2.5* | 0/0/15 | - | |
| 5.0 | 0/15/15 | - | |
| 6.5 | 1/15/15 | 2h | 8.2 |
| 7.5 | 6/15/15 | 1-2h | (7.5 - 9.0 |
| 10.0 | 11/15/15 | 1-2h | s = 1.2 |
| 11.0 | 15/15/15 | 30-2h | |
| | | Female dogs | |
| 1.0* | 0/ 0/1 | - | |
| 2.0*** | 0/ 2/2 | - | "approx. 5" |
| 5.0*** | 1/ 1/2 | 4h | |
| 10.0 | 1/ 1/1 | 4h | |

*highest no-effect dose
**1st number = no. of animals that died
2nd number = no. of animals exhibiting signs of toxicity
3rd number = no. of animals on test
***test compound emulsion vomited

# Acute Oral Toxicity in Guinea Pigs

Fiche/Master ID 00071872

Crawford, C.R.; Anderson, R.H. (1973) The Acute Oral Toxicity of Di-Syston Technical to Guinea Pigs: Report No. 39113. (Unpublished study received Dec. 15, 1976 under 3125-58; submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL: 095640-F)

Test Chemical:

Technical Di-Syston 95%.

Experimental Protocol:

Groups of 3 male (440-525 gm) and 3 female guinea pigs (394-491 gm) were fasted for a period of 20 hours prior to oral dosing with various levels of Di-Syston in 20% ethanol in propylene glycol. The animals were observed for pharmacotoxic symptoms for a period of 14 days. The $LD_{50}$ and 95% confidence limits were determined according to the method of Weil (C.S. Weil, Biometrics, 8, 1952).

Results:

The authors stated that the animals responded in a dose-related manner, and the intoxication symptoms manifested were typical of those associated with cholinesterase inhibition. The $LD_{50}$ was determined to be 8.9 (5.6-14.1) and 12.7 (8.1-20.1) mg/kg for males and females respectively. The death occurred within the first 12 hours.

Toxicity Category:

On the basis of the acute oral toxicity, this chemical is assigned to Toxicity Category I.

Core Classification:

Core Supplementary data. Experimental details and raw data were not provided to ascertain the accuracy of the $LD_{50}$ values reported. Inadequate number of animals was tested.

# Acute Toxicity of Di-Syston to Weanling and Adult Rats

Fiche/Master ID 05004291

Brodeur, J.; DuBois, K.P. (1963) Comparison of acute toxicity of anticholinesterase insecticides to weanling and adult male rats. Pages 509-511, In Proceedings of the Society for Experimental Biology and Medicine. Vol. 114. New York: Academic Press.

Test Chemical:

Di-Syston

Experimental Protocol:

Twenty-five Weanling (23 day old, 50 to 60 gm) and 30 adult (200 to 300 gm) male Holtzman rats were used in this study. Di-Syston was dissolved in 20% ethanol in propylene glycol, and the concentrations were adjusted so that each animal received an amount equivalent to 0.2% of the body weight to the weanlings and an amount equivalent to 0.1% to the adult. The test chemical was administered intraperitoneally. The animals were observed for 14 days after administration of the test chemical. The $LD_{50}$ and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (J. Pharm. Exp. Therap., 1949, 95, 99).

Results:

According to the authors, after administration of the test chemical, symptoms typical of those resulting from the cholinesterase inhibition were observed.

The $LD_{50}$ of Di-Syston was determined to be 5.4 (5.0-5.8) mg/kg and 9.4 (8.5-10.3) mg/kg for weanling and adult male rats respectively.

Discussion and Conclusions:

Di-Syston is about twice as toxic to weanling as it is to adult male rats. The authors in their discussion of the findings indicated that "the mechanisms responsible for age difference in susceptibility to cholinergic organic phosphates were not clear. Di-Syston and most of the compounds which exhibit age differences in toxicity must undergo metabolic conversion to potent anticholinergic agent. However, the rate of conversion to an active metabolite is slower in the livers of weanlings than in adults (Murphy and DuBois, J. Pharm. Exp. Therap., 1958, 124, 194). Differences in rate of

activation, therefore, cannot account for the results obtained.

"Previous studies (DuBois and Puchala, Soc. Exp. Biol. and MED, 1966, 107, 908) have provided substantial evidence that some phosphorothioates are detoxified by the catalytic action of microsomal enzymes and that the toxicity of some phosphorathioates and dithioates is governed to various degrees by these metabolic reactions. It has been well established that the microsomal enzymes that catalyze the drug metabolism develop after birth of some species of experimental animals (Fouts and Adamson, 1959, 129, 897). It appears that the rate and extent of detoxification of certain anticholinesterase insecticides more closely parallels the liver microsome enzyme levels than does the activation reaction which the compound must also undergo to exhibit anticholinesterase activity. It appears that activation of the compounds requires only a small fraction of the total amount of microsome oxidase activity in the adult liver. Thus rate of detoxification would tend to have a marked influence on toxicity of the compounds."

Core Classification:

Supplementary data. Experimental details and raw data were not provided.

003958

# The Acute Oral Toxicity of Di-Syston Metabolites to Rats

Fiche/Master ID 00071873

Crawford, C.R.; Anderson, R.H. (1974) The Acute Oral Toxicity of Several Di-Syston Metabolites to Female and Male Rats: Report No. 39687. (Unpublished study received Dec. 15, 1976 under 3125-58; submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:095640-G)

Test Chemical(s):

Technical Di-Syston, 96% batch #1050329, Di-Syston sulfoxide, Di-Syston sulfon, thiol Systox, thiol systox sulfoxide and thiol systox sulfon.

Experimental Protocol:

According to the authors "fasted female rats weighing 200-260 gm. and fasted male rats weighing 270-325 gm. were used in this study. The animals were fasted approximately 20 hours before any compounds were administered. Di-Syston Technical and the metabolites were either diluted with or dissolved in a solution of 20% ethanol-80% propylene glycol to a final concentration so that each animal received the required dose in a volume equivalent to 0.1% body weight. Four groups of females and one group of males were intubated with a slightly curved stainless 3 inch animal feeding needle. Each group consisted of 4 animals. The female animals were exposed to graded doses of the compounds. To measure sex susceptibility male animals were exposed only to the $LD_{50}$ dose established for each compound to female animals. Symptoms and mortality were recorded for 14 days. The acute $LD_{50}$ and 95% confidence limits were calculated according to a method of Weil (Carrol S. Weil, Biometrics, 8, 249, 1952)."

Results:

The results are presented in Table 1. According to the authors the intoxication symptoms were typical of those associated with inhibition of cholinesterase.

Table 1. Acute Oral Toxicity of Several Di-Syston Metabolites.

| Compound | Sex | Dose mg/kg | Observations Deaths/ Symptoms/No. Exposed | Symptoms Start (Min) | Symptoms End (Hr) | Time of Death (Hr) | $LD_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|---|
| Di-Syston Technical | Female | 0.5 | 0/4/4 | 60 | 3 | – | |
| | | 1.0 | 0/4/4 | 60 | 3 | – | |
| | | 2.0 | 2/4/4 | 30 | 4 | 3 | |
| | | 4.0 | 4/4/4 | 30 | 4 | 1 | 2.0(1.3-3.0) |
| Di-Syston Technical | Male | 2.0 | 0/0/4 | – | – | – | >2.0 |
| Di-Syston Sulfoxide | Female | 1.1 | 0/1/4 | 60 | 4 | – | |
| | | 1.5 | 0/4/4 | 60 | 4 | – | |
| | | 2.0 | 3/4/4 | 60 | 4 | 3 | |
| | | 2.7 | 4/4/4 | 50 | – | 1 | 1.7(1.5-1.9) |
| Di-Syston Sulfoxide | Male | 1.7 | 0/0/4 | – | – | – | >1.7 |
| Di-Syston Sulfone | Female | 0.85 | 0/4/4 | – | – | – | |
| | | 1.13 | 2/4/4 | 60 | 2 | – | |
| | | 1.50 | 3/4/4 | 60 | – | 1 | |
| | | 2.00 | 4/4/4 | 60 | – | 1 | 1.24( )* |
| Di-Syston Sulfone | Male | 1.24 | 0/0/4 | – | – | – | >1.24 |
| Thiol systox | Female | 0.85 | 0/4/4 | 60 | 2 | – | |
| | | 1.13 | 2/4/4 | 60 | 3 | – | |
| | | 1.50 | 3/4/4 | 60 | 3 | | |
| | | 2.00 | 4/4/4 | 30 | – | | 1.17(0.96-1.4) |
| Thiol systox | Male | 1.17 | 0/0/4 | – | – | | >1.17 |

Table 1. Acute Oral Toxicity of Several Di-Syston Metabolites. Cont'd

| Compound | Sex | Dose mg/kg | Observations Deaths/ Symptoms/No. Exposed | Symptoms Start (Min) | Symptoms End (Hr) | Time of Death (Hr) | $LD_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|---|
| Thiol systox Sulfoxide | Female | 0.85 | 0/4/4 | 60 | 3 | - | |
| | | 1.13 | 0/4/4 | 60 | 3 | - | |
| | | 1.50 | 4/4/4 | 75 | - | 1.75 | |
| | | 2.00 | 4/4/4 | 75 | - | 1.75 | 1.24( )* |
| Thiol systox Sulfoxide | Male | 1.24 | 0/4/4 | 90 | 3 | - | >1.24 |
| Thiol systox Sulfone | Female | 0.85 | 0/4/4 | 120 | 2 | - | |
| | | 1.13 | 2/4/4 | 60 | 3 | 3-4 | |
| | | 1.50 | 4/4/4 | 60 | - | 2-3 | |
| | | 2.00 | 4/4/4 | 30 | - | - | 1.10(0.95-1.27) |
| Thiol systox Sulfone | Male | 1.10 | 0/4/4 | 90 | 3 | - | >1.10 |

*Confidence limits impossible to calculate due to all-or-none nature of data.
**This table is taken from the original report.

Conclusions:

The five Di-Syston metabolites tested were more toxic to female rats than the parent compound Di-Syston. Female rats were more susceptible to Di-Syston and its metabolites than male rats.

Core Classification:

Core-supplementary data.

Potentiation Study in the Rat

Fiche/Master ID 00011448

Johnston, C.D. (1966) GS 13005 25W: Potentiation Studies with Other Organophosphate Insecticides in the Rat: Addendum. (Unpublished study received Oct. 1, 1969 under 0F0892; prepared by Woodard Research Corp., submitted by Geigy Chemical Corp., Ardsley, N.Y.; CDL:091535-X)

Test Chemical:

Di-Syston 99%, and GS 13005 25W (currently known as Supracide or Methidathion).

Experimental Protocol:

This report is an addendum to another report dated December 7, 1965 described results of experiments conducted to assess the hazard of potentiation from combinations of GS 13005 with other anticholinesterase insecticide. The author indicated that the experimental procedures and conditions were the same as described in the original report. Doses of GS 13005 25W were in terms of formulation, and the $LD_{50}$ value used was 50 mg/kg. Adult female albino rats wee used in this study. Polyethylene glycol was used to dissolve the test chemicals. The method of Horn described in "simplified $LD_{50}$ or ($ED_{50}$) calculations", Biometrics, 12, 311 (1956), was used to calculate the $LD_{50}$ values.

Results:

The $LD_{50}$ data for Di-Syston are presented in Table 1.

Table 1: Acute Oral Toxicity of Di-Syston in the Female Rat

| Dose ml/kg | Mortality Days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 hour | 1 | 2 | 3 | 4 | 5 | 7 |
| $10.0 \times 10^{-3}$ | 5/5 | | | | | | 5/5 |
| $4.64 \times 10^{-3}$ | 0/5 | 1/5 | 1/5 | 1/5 | 2/5 | 3/5 | 3/5 |
| $2.15 \times 10^{-3}$ | 0/5 | 0/5 | 0/5 | 1/5 | | | 1/5 |
| $1.00 \times 10^{-3}$ | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 1/5 | 1/5 |

The $LD_{50}$ was determined to be $3.48 \times 10^{-3}$ ml/kg, equivalent to 3.9 (2.1-7.4) mg/kg.

The potentiation data are presented in Table 2.

Table 2: Potentiation of the Acute Oral Toxicity of Di-Syston and GS 13005

| GS 13005 $LD_{50}$ (mg/kg) | Di-Syston $LD_{50}$ (mg/kg) | Mortality | | |
|---|---|---|---|---|
| | | Fraction of $LD_{50}$ GS 13005 25W | 1/2 | 1/4 |
| | | Fraction of $LD_{50}$ Di-Syston | 1/2 | 1/4 |
| 50 | 3.9 | | 20/20 | 3/20 |

Conclusions:

The interaction of Di-Syston and GS 13005 25W can be considered as an additive effect and not a potentiation effect.

Core Classification:

Core supplementary data.

# Potentiation Studies in the Rat

Fiche/Master ID 00075432

Johnston, C.D. (1963) Imidan Potentiation Studies in the Rat with Other Organophosphate Insecticides. Unpublished study, including letters dated Jun. 4, 1963 from C.D. Johnston to A.B. Lindquist and dated Dec. 11, 1963 and Jan. 28, 1965 from C.D. Johnston to George D. Meyding, received Apr. 8, 1976 under 476-2167; prepared by Woodard Research Corp., submitted by Stauffer Chemical Co., Richmond, Calif.; CDL:233439-I)

Test Chemical:

Di-Syston 99%, and technical Imidan (phthalimidomethyl-O,O-dimethyl phosphorodithioate).

Experimental Protocol:

According to the author, groups of five female adult albino rats (150-247 gm) were fasted overnight, then given single doses, by stomach tube, of the test compound. Initially, the acute oral $LD_{50}$ of each insecticide was estimated using the procedure described by Horn (Biometrics, Vol. 12, pg. 311, 1956). Dose schedules provided in Horn's publication permit estimation of the $LD_{50}$ (with confidence limits) from the mortality ratios.

The lethality of the combination of Di-Syston plus Imidan was determined, by administering both materials together at a fraction of each estimated $LD_{50}$. The mortality ratio resulting from the combined compounds furnished a basis to judge whether the toxicity of the two was additive or greater than additive, i.e., potentiated.

After dosing, the rats were returned to their cages, and observed for the rest of the testing day. Food and water were provided. Daily observations for mortality were made for seven days, at which time survivors were sacrificed. The compounds were tested as corn oil solutions.

For the first potentiation experiments the mixture of the two components was to contain one-half the $LD_{50}$ of Di-Systor plus one-half that of Imidan. The solvent for the two components was that used to determine the single $LD_{50}$ values.

Groups of 20 female rats each were used and the general conditions noted above were maintained. Subsequent groups of 20 female rats each were tested with the two component mixture at one-fourth of the respective $LD_{50}$.

Results:

The $LD_{50}$ for Di-Syston was determined to be 3.32 (2.57-4.28) mg/kg and for Imidan 224 (176-286) mg/kg. The results of the $LD_{50}$ determination are presented in Tables 1 and 2.

The $LD_{50}$ of Di-Syston was decreased when the two chemicals were tested in equitoxic mixture of one-half of the $LD_{50}$. The decrease in $LD_{50}$ was about one half. However, when the two chemicals were tested at one fourth the $LD_{50}$ value of each, no mortality occurred. The results of the potentiation testing are presented in Table 3.

Table 1: Acute Oral $LD_{50}$ of Di-Syston in the Female Rat

| Dose mg/kg | 1 hour | Mortality Days 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 6.81 | 5/5 | | | | | | | |
| 4.64 | 5/5 | | | | | | | |
| 3.16 | 0/5 | 1/5 | | | | | | 1/5 |
| 2.15 | 0/5 | 1/5 | | | | | | 1/5 |
| 1.47 | 0/5 | | | | | | | 0/5 |

$LD_{50}$: 3.32 (2.57-4.28) mg/kg

Table 2: Acute Oral $LD_{50}$ of Imidan in the Female Rat

| Dose mg/kg | 1 hour | Mortality Days 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 464 | 2/5 | 5/5 | | | | | | 5/5 |
| 316 | 2/5 | 4/5 | | | | | | 4/5 |
| 215 | 1/5 | 2/5 | | | 3/5 | | | 3/5 |
| 147 | 0/5 | | | | | | | 0/5 |

$LD_{50}$: 224 (176-286) mg/kg

Table 3: Acute Oral Toxicity of Di-Syston in Combination With Imidan

| $LD_{50}$ of Di-Syston | $LD_{50}$ of Imidan | Mortality 1/2 $LD_{50}$ Imidan Plus 1/2 $LD_{50}$ Di-Syston | 1/4 $LD_{50}$ Imidan Plus 1/4 $LD_{50}$ Di-Syston |
|---|---|---|---|
| 3.3 | 224 | 18/20 | 0/20 |

Conclusion:

The interaction of Di-Syston and Imidan can be considered as an additive effect rather than a true potentiation.

Core Classification:

Core supplementary data.

Potentiation of the Acute Toxicity

Fiche/Master ID 00069351

DuBois, K.P. (1957) The Acute Toxicity of Di-Syston in Combination with Other Organic Phosphate to Rats: Report No. 1995. (Unpublished study received Nov. 20, 1957 under 3125-58; prepared by Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:100153-F)

Test Chemical(s):

Di-Syston, unspecified formulation and purity, and other organic phosphates such as parathion, systox, malathion, EPN, methyl parathion, guthion, trithion and diazinon.

Experimental Protocol:

According to the author, young adult female Sprague-Dawley rats (200-250 gm) were used in this experiment. Prior to the potentiation tests the acute oral $LD_{50}$ of each compound was determined. For the purpose of $LD_{50}$ determination, several doses of each compound were administered. The $LD_{50}$ values were calculated from the mortality data by the logarithm probability method. For the purpose of potentiation test, the animals were simultaneously dosed orally with one-half of the $LD_{50}$ of Di-Syston plus one-half of the $LD_{50}$ of parathion, systox, malathion, EPN, methyl parathion, guthion, trithion, or diazinon. All of the compounds except malathion and trithion were dissovled in 10% ethanol in propylene glycol. Malathion was administered undiluted. Trithion was dissolved in 20% ethanol in propylene glycol. In all cases the quantity of the dosing solution was less than 1% of the body weight. Animals were observed for a period of 10 days after treatment.

Results:

No raw data were submitted. The results were reported as summary tables. The results of the $LD_{50}$ determination are presented in Table 1.

Table 1: Acute Oral Toxicity of Several Organic Phosphates To Female Rats

| Organic Phosphate | No. of Animals | Approx. $LD_{50}$ (mg/kg) |
|---|---|---|
| Di-Syston | 36 | 2.6 |
| Parathion | 31 | 3.8 |
| Methyl Parathion | 32 | 8.0 |
| Guthion | 40 | 12.0 |
| EPN | 40 | 9.0 |
| Systox | 34 | 3.7 |
| Malathion | 32 | 1400 |
| Trithion | 35 | 6.0 |
| Diazinon | 45 | 400 |

The data indicated that [illegible]e oral administration of two component mixtures of e[illegible]toxic proportion of Di-Syston and other organophosphates [illegible] not affect the $LD_{50}$ of either component. The effect was considered to be strictly an additive effect. The results of the potentiation experiment are shown in Table 2.

Table 2: Oral Toxicity of Di-Syston Plus Other Organic Phosphates to Rats

| Di-Syston (mg/kg) | 2nd Organic Phosphate | Dose (mg/kg) | Mortality | % Mortality |
|---|---|---|---|---|
| 1.3 | Parathion | 1.9 | 11/20 | 55 |
| 1.3 | Systox | 1.85 | 9/20 | 45 |
| 1.3 | Malathion | 700.00 | 11/20 | 55 |
| 1.3 | EPN | 4.5 | 8/20 | 40 |
| 1.3 | Methyl Parathion | 4.0 | 4/20 | 20 |
| 1.3 | Guthion | 6.0 | 3/20 | 15 |
| 1.3 | Trithion | 3.0 | 9/20 | 45 |
| 1.3 | Diazinon | 200.00 | 10/20 | 50 |

Conclusions:

The acute toxicity of Di-Syston is not potentiated by other organophosphates when administered in two-component mixtures of equitoxic proportions.

Core Classification:

Supplementary data. No experimental details were provided and the data were reported as summaries.

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Some of the materials included in this review were taken from the original report.

# Potentiation Studies in the Rat

Fiche/Master ID 00006794

Scott, W.J.; Beliles, R.P. (1965) Phosalone: Potentiation Studies in the Rat with Marketed Pesticides. (Unpublished study received Dec. 15, 1966 under 7G0575; prepared by Woodard Research Corp., submitted by Chipman Chemical Co., Inc., Burlingame, Calif.; CDL:0970729-I)

Test Chemical:

Di-Syston 99%, and Phosalone described as brownish white granular powder.

Experimental Protocol:

According to the author the oral $LD_{50}$'s of pesticides were first determined using the method of H. J. Horn (Biometrics, 12:311, (127-217 gm) and each received a logarithmically spaced dose of test material. Di-Syston was dissolved or suspended in polyethylene glycol and administered by stomach tube. For this reason an $LD_{50}$ determination using each suspending agent was performed on Phosalone.

Following compound administration, all rats were observed closely for several hours for toxicity signs. The rats were observed periodically for the remainder of the working day and daily thereafter for a period of seven days, or until death. Animals that died during the observation period and representative survivors sacrificed at the end of the observation period were subjected to gross necropsy.

To determine any possible potentiation, twenty female rats were given a solution containing one-half the $LD_{50}$ of Di-Syston plus one-half the $LD_{50}$ of phosalone by stomach tube. Following compound administration all rats were observed closely for toxicity signs as before. If 15 or more animals died it was judged that potentiation existed and another group of twenty rats were administered one-half of the previous dose of Di-Syston and Phosalone. This procedure was continuted until less than 15 animals died.

Results:

The $LD_{50}$ for Di-Syston was determined to be 3.61 (2.68-4.86) mg/kg in corn oil and for Phosalone 153 (120-194) mg/kg in polyethylene glycol, and 207 (172-250 mg/kg) in corn oil. The results of the $LD_{50}$ determination for Di-Syston and Phosalone are presented in Tables 1 and 2.

Under the test conditions, Di-Syston was shown to potentiate or be potentiated by the action of Phosalone. Di-Syston continued to potentiate or be potentiated by the action of Phosalone at a fraction of one-sixteenth the $LD_{50}$ of each chemical. The results of the potentiation study is presented in Table 3.

In the case of Di-Syston the only toxic sign evident at the lowest level was slight depression. At higher levels additional signs were evident including tremors, ataxia, lacrimation, salivation, diarrhea and prostration. No visible lesions were observed in gross necropsy.

In the case of Phosalone depression was the first toxic sign to appear and was evident at all levels. Tremors, exophthalmos, lacrimation and diarrhea were evident in rats receiving doses approaching the $LD_{50}$ or higher. At necropsy, sporading occurrence of pulmonary hemorrhage was observed.

Table 1: Acute Oral Toxicity of Di-Syston to Female Rats

| Dose ml/kg | Conc. mg/ml | Mortality (Days) 1 hr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Cumulative Mortality | Mean Body Wt.(g) Day 0 | Day 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6.81 | 0.990 | | | 3 | | 2 | | | | 5/5 | 149 | |
| 4.64 | 0.990 | | 3 | 1 | | | | | | 4/5 | 143 | 144 |
| 3.16 | 0.990 | | 1 | | | | | | | 1/5 | 146 | 168 |
| 2.15 | 0.990 | | | 1 | | | | | | 1/5 | 154 | 178 |

Solvent: corn oil
$LD_{50}$ : 3.61 (2.68-4.86) mg/kg

Table 2: Acute Oral Toxicity of Phoslone to Female Rats

| Dose mg/kg | Conc. mg/ml | Mortality (Days) 1 hr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Cumulative Mortality | Mean Body Wt.(g) Day 0 | Day 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 316 | 20 | 5 | | | | | | | | 5/5 | 156 | |
| 215 | 20 | | 2 | 1 | 1 | | | | | 4/5 | 147 | 185 |
| 147 | 20 | | 1 | 1 | 1 | | | | | 3/5 | 159 | 191 |
| 100 | 20 | | | | | | | | | 0/5 | 151 | 182 |
| 68.1 | 20 | | | | | | | | | 0/5 | 161 | 194 |

Solvent: polyethylene gylcol
$LD_{50}$ : 153 (120-194) mg/kg

Table 3: Potentiation of the Acute Toxic Effects of Di-Syston and Phosalone in Albino Rats

| Fraction of $LD_{50}$* | | | Mortality (Days) | | | | | | | Cumulative | Mean Body Wt.(g) Day | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phosalone | Di-Syston | 1 hr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Mortality | 0 | 7 |
| 1/2 | 1/2 | | | 13 | 3 | 1 | | | | 17/20 | 186 | 203 |
| 1/4 | 1/4 | | 9 | 8 | | | 2 | | | 19/20 | 174 | 182 |
| 1/8 | 1/8 | | 12 | 6 | | | | 1 | | 19/20 | 166 | 178 |
| 1/16 | 1/16 | | 17 | 3 | | | | | | 20/20 | 168 | - |
| 1/32 | 1/32 | | | | | | | | | 0/20 | 161 | 209 |

* Solvent = corn oil

Conclusions:

Di-Syston can potentiate or be potentiated by Phosalone.

Core Classification:

Core Minimum data

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The experimental protocol and tables were taken from the original report.

# Acute Intraperitoneal Toxicity in Rats, Mice and Guinea Pigs

Fiche/Master ID 00069347

Bombinski, T.J.; DuBosi, K.P. (1957) The Acute Mammalian Toxicity and Pharmacological Actions of Di-Syston: Report No. 1732. (Unpublished study received No. 20, 1957 under 3125-58; prepared by Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:100153-B)

Test Chemical:

Technical Di-Syston with unspecified purity.

Experimental Protocol:

Di-Syston was intraperitoneally administered to male and female Sprague-Dawley rats, male and female Carworth Farms Mice and male guinea pigs. Di-Syston was dissolved in 10% ethanol in propylene glycol. The strength of the solutions were always adjusted so that less than 0.3% of the body weight was administered to rats, and volume corresponding to less than 1% of the body weight was given to mice. The animals were housed in air-conditioned rooms at 65°F to 75°F and fed Rockland Rat Diet. The animals were observed for a period of 10 days. The $LD_{50}$ values were calculated from the mortality data using the logarithm-probability method.

Results:

The data were presented in a summary table as follows:

| Species | Sex | No. of Animals | Approx. $LD_{50}$ (mg/kg) |
|---|---|---|---|
| Rats | Male | 35 | 10.5 |
| | Female | 43 | 2.0 |
| Mice | Male | 35 | 1.1 |
| | Female | 40 | 6.5 |
| Guinea Pigs | Male | 30 | 7.0 |

According to the author, the intoxication symptoms were qualitatively similar to those result from poisoning by other cholinergic organic phosphates.

After the administration of doses near the $LD_{50}$, symptoms became evident in about one-half hour. The initial effects consisted of excitability, salivation, lacrimation, defecation, and muscular fasciculations. After lethal doses, these symptoms were followed by convulsive seizures, prostration and cessation of respiration. The time of onset and duration of the symptoms were dependant upon the dose of Di-Syston. Following lethal doses, death usually occurred within 48 hours but with doses around the $LD_{50}$ death was sometimes delayed for several days.

Discussion and Conclusions:

A sex difference in susceptibility to Di-Syston was observed. Male rats were 5 times less susceptible than female rats. On the other hand, a difference of a similar magnitude but in the opposite direction was observed in mice. The comparison of the toxicity of the compound given orally (a report by the same authors) and intraperitoneally to rats indicates that Di-Syston is rapidly absorbed from the gastrointestinal tract. Experimental details and raw data were not provided.

Core Classification:

Not applicable. The data are considered of supplemental nature.

Acute Dermal Toxicity in the Rat

Fiche/Master ID 00043213

DuBois, K.P. (1957) The Dermal Toxicity of Di-Syston to Rats: Report No. 2063. (Unpublished study received Jan. 23, 1958 under unknown Admin. No.; prepared by Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:109216-8)

Test Chemical(s):

Technical Di-Syston, emulsifiable concentrates 2 and 8 pounds per gallon, and 50% [redacted] and [redacted] powders.

Experimental Protocol:

Technical Di-Syston (unspecified purity) was dissolved in xylene and applied to the shaved back of adult male Sprague-Dawley rats. The application sites were ~24 mm in diameter. The 50% [redacted] and [redacted] powders were diluted with a 2% aqueous solution of methyl cellulose. The animals were observed for a period of ten days after treatment since death or apparent complete recovery occurred within this period of time. No more experimental details were provided.

Results:

According to the author, the $LD_{50}$ values were calculated from the mortality data using the logarithm-probability method, based on the actual Di-Syston contents. The $LD_{50}$ values for all the formulation tested are shown in Table 1 below. No more results were provided.

Table 1: Relative Acute Dermal Toxicity of Different Di-Syston Formulation to Male Rats

| Test Chemical | No. of Animals | Approximate $LD_{50}$ (mg/kg) | |
|---|---|---|---|
| | | Formulation | Based on Di-Syston Cont. |
| Technical | 35 | 20 | 20 |
| EC 2 lbs/gal | 30 | 84 | 21 |
| EC 8 lbs/gal | 34 | 20 | 20 |
| 50% [redacted] powder | 32 | 70 | 35 |
| 50% [redacted] powder | 30 | 60 | 30 |

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Conclusions:

The acute dermal $LD_{50}$ of technical Di-Syston is approximately 20 mg/kg.

Toxicity Category:

Based on the acute dermal toxicity, the test chemicals are assigned to Toxicity Category I.

Core Classification:

Supplementary data. The study was performed on males only. Dose levels and the number of animals per group were not specified. Symptoms of intoxication were not reported. It was not clear from the report whether a control group was included.

Fiche Master ID 000000000

Mihail, F. (1976). S 276 (Disyston Active Ingredient) Acute Toxicity Studies. Report No. 7062b prepared by Bayer AG, Institut Fur Toxikologie for Mobay Chemical Corporation. June 12, 1978.

Test Chemical:

Di-Syston technical 94.4%.

Experimental Protocol:

Forty male and 45 female Wistar rats weighing between 170 and 230 grams were obtained from Winklemann, Borchen. The number of animals per dose group was as follows: 5 males at the 5.0 and 20.0 mg/kg dose levels; 10 males at 10.0, 15.0 and 17.5 mg/kg dose levels; 10 females at the 2.5, 3.0, 3.5 and 5.0 mg/kg dose levels; and 5 females at the 10.0 mg/kg level.

The animals were housed in Makrolon® cages (number per cage not specified) and provided food (Altromin R Small Animal Diet) and water ad libitum. The method of Noakes and Sanderson (1969, Brit, J. Indust. Med., 26:59) was used for this study. The day before dosing the dorsal fur was removed from each animal. The test compound was mixed into a paste with water and Cremophor EL, applied to the intact dorsal skin, and held in place with adhensive plaster sleeves. Twenty-four hours later the sleeves were removed, the test site washed with soap and water, and the animals observed for 14 days.

Necropsy was performed on rats which died during the study. The median lethal dose was calculated by the method of Litchfield and Wilcoxon.

RESULTS:

Toxic signs ("impairment of general health conditions," muscle twitching, clonic cramps, and breathing disorders) were observed in all animals at all dose levels at unspecified times after dosing. The number of deaths and time to death for animals at the various dose levels are shown in Table 1. After 14 days, the median lethal dose and 95 percent confidence interval was 15.9 mg/kg (14.8 - 17.0) for males and 3.6 mg/kg (3.1 - 4.0) for females. At autopsy, pale livers and kidneys were observed in rats which died during the study.

TABLE 1. Acute Dermal Toxicity Results

| Dose (mg/kg) | Toxicological Result* | Time of Death Days | $LD_{50}$** (mg/kg) |
|---|---|---|---|
| | Male Rats | | |
| 5.0 | 0/ 5/ 5 | - | |
| 10.0 | 0/10/10 | - | 15.9 |
| 15.0 | 3/10/10 | 2-6 | (14.8-17.0) |
| 17.5 | 8/10/10 | 1-5 | |
| 20.5 | 5/ 5/ 5 | 1-3 | s = 1.2 |
| | Female Rats | | |
| 2.5 | 0/10/10 | - | |
| 3.0 | 2/10/10 | 3-5 | 3.6 |
| 3.5 | 7/10/10 | 1-5 | (3.1-4.0) |
| 5.0 | 9/10/10 | 1-6 | |
| 10.0 | 5/ 5/ 5 | 1 | S = .2 |

*1st number = no. of animals that died.
2nd number = no. of animals exhibiting signs of toxicity.
3rd number = no. of animals on test.

**95 percent confidence interval presented in parenthesis.

DISCUSSION:

Several deficiencies were noted in the study design and in data reporting. Individual animal body wegihts were not determined during the 14-day observation period, the initial body weight range (170 to 230 grams) was not entirely within the adult rat body weight range (200 to 300 g) recommended in the Pesticide Assessment Guidelines (USEPA, 1982), and the dose application method was poorly described. However, none of these shortcomings is sufficient to jeopardize the study results.

CONCLUSIONS:

Under the conditions of this study with Wistar rats, the acute dermal $LD_{50}$ was 15.9 mg/kg (14.8 - 17.0) in males and 3.6 mg/kg (3.1 - 4.0) in females

CORE CLASSIFICATION:

Minimum Data.

TOXICITY CATEGORY:

On the basis of acute dermal toxicity the chemical is assigned to "Toxicity Category I".

The Acute Inhalation Toxicity of Di-Syston to Rats and Mice

003958

Fiche/Master ID 00069349

Doull, J. (1957) The Acute Inhalation Toxicity of Di-Syston to Rats and Mice: Report No. 1802. (Unpublished study received Nov. 20, 1957 under 3125-58; prepared by Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:100153-D).

Test Chemical:

Technical Di-Syston with unspecified purity.

Experimental Protocol:

Forty male adult Sprague-Dawley rats weighing 250-300 gm, and 60 female Carworth Farms mice weighing about 20 gm were divided into groups of 5 rats or 10 mice each and exposed to various aerosol concentrations in a 200-liter dynamic flow gassing chamber for one hour. The exposure time was reduced for one group to 30 min. The Di-Syston aerosols were generated from xylene solutions of technical Di-Syston by means of Vaponephrine Nebulizer operated at an air pressure of 15 pounds per sq. inch. The average particle size was between 1 and 3 microns in diameter. The flow rate was maintained at one chamber volume per minute. The concentrations of the test chemical was continuously monitered. The animals were individually caged during exposure and were observed for pharmacctoxic signs for 10 days after exposure.

Results:

The authors stated that "the concentrations of Di-Syston used in these studies were sufficient to produce severe symptoms and occasional deaths during the exposure period. The symptoms were similar to those seen following the administration of Di-Syston by other routes of administration and included increased salivation, urination, lacrimation, and defecation together with muscular twitching, fibrillations and incoordination of gait. Animals that died during the exposure usually exhibited convulsive seizure prior to death. Although most of the deaths which occurred following the gassing exposure took place within the first 24 hours, one of the rats did not die until 6 days after the Di-Syston exposure."

The $LC_{50}$ was determined to be 0.05 and 0.2 mg/l for mice and rats respectively.

Toxicity Category:

On the basis of the acute inhalation toxicity, Di-Syston is assigned to Toxicity Category I.

Core Classification:

Core Supplementary data. However, further testing may not be required since the chemical has been assigned Toxicity Category I.

# Acute Oral Toxicity in Hens

Fiche Master ID 000000000

Hixon, E.J.: Acute oral toxicity of ®DI-SYSTON technical in hens. An unpublished report (No. 341) prepared by the Environmental Health Research Institute of Mobay Chemical Corporation, Stilwell, Kansas. Study No. 82-018-01, dated October 25, 1982.

Test Chemical:

Di-Syston technical 97.8%.

Experimental Protocol:

1. A total of 25 adult White Leghorn hens, 17 months old, ranging in weight from 0.94 - 1.59 kg, were held in quarantine for six days prior to study initiation. Hens were divided into 5 groups and were administered orally a single dose of undiluted DI-SYSTON at levels of 12.5, 20, 32, 51, or 82 mg/kg.

2. Hens were fed "Purina Layena" and water ad libitum and were housed in stainless steel cages. Room temperature was kept at 69 - 74°F and relative humidity was 35 to 55 percent, with 12 hours light-dark cycles.

3. Hens were observed for signs of toxicity of 0.5, 1 and 4 hours after treatment, and at least once per day for 14 days. Body weights were determined on days 0, 7, and 14.

4. The $LD_{50}$ value was calculated using the Probit method. All survivors on day 14 were sacrificed by cervical dislocation, and necropsied.

RESULTS:

1. Signs of toxicity observed included staggering gait, tremors, lacrimation and salivation.

2. No significant change in mean body weights was observed in surviving hens.

3. Mortality, occurred within 1 hour to 1 day after treatment. $LD_{50}$ and confidence limits are shown in the following table.

| Dose (mg/kg) | Mortality | $LD_{50}$ mg/kg (95 percent confidence limit) |
|---|---|---|
| 12.5 | 0/5 | |
| 20.0 | 2/5 | |
| 32.0 | 2/5 | 27.5 (18.6 - 41.0) |
| 51.0 | 5/5 | |
| 82.0 | 5/5 | |

4. Gross examination of hens that died after treatment showed fluidfilled gastrointestinal tract, congested intestinal mucosa, dark red foci in the liver, and froth in the crop. It was reported that "Gross findings in hens that were sacrificed were considered incidental."

CONCLUSIONS:

The acute oral $LD_{50}$ of undiluted Technical DI-SYSTON in adult White Leghorn hens was 27.5 mg/kg, with 95 percent confidence limits of 18.6 to 41.0 mg/kg.

CORE CLASSIFICATION: Supplemental information.

Acute Delayed Neurotoxicity Hens

Fiche Master ID 000000000

Hixson, E.J. (1983) Acute delayed neurotoxicity study on disulfoton. Toxicology Report No. 365 (Study No. 82-418-01) prepared by Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, MO, and dated March 7, 1983.

Test Chemical:

Disulfoton technical 97.8%.

Experimental Protocol:

Forty retired White Leghorn laying hens, weighing between 1.03 and 1.61 kg, were used in this study. The animals were obtained from Colonial Poultry Farms, Pleasant Hill, MO. Animals were observed at least once daily for at least one week prior to study initiation and randomly assigned to numbered cages. Animals were individually housed in stainless steel cages with wire mesh floors. Tapwater and Purina Layena were provided ad libitum. The animal room was maintained at 69 to 78°F and 45 ± 10 percent relative humidity with a 12-hour light/dark cycle.

The birds were divided into 4 groups. One group, containing 20 hens, was treated orally, using Hamilton syringes, with undiluted test material at 30 mg/kg body weight. These birds also were treated with a 0.5 mg/kg i.m. dose of atropine 10 minutes before test material treatment, and with a 12.5 mg/kg i.m. dose of 2-PAM, 30 minutes after test material dosing. A second group (antidote control) of 5 birds received atropine and 2-PAM at the same dosages and schedule but did not receive any test material. These two groups were dosed on two separate occasions 21 days apart. A third group (positive control) of 10 birds received 500 mg/kg of undiluted tri-o-cresyl phosphate orally. The last group of 5 birds was untreated. Dose volumes for the first three groups were calculated on the basis of body weight determined on the day of dosing.

The hens were obsreved for signs of neurotoxicity daily for a total of 42 days. Body weights were measured and feed consumption estimated twice weekly throughout the study. After 42 days, the hens were anesthetizes with an unspecified barbituate and sacrificed by formalin infusion. At necropsy, gross examination was performed and the following tissues were removed and prepared for histopathologic evaluation: sciatic nerve, spinal cord (cervical, thoracic, lumbar), brain (mid-brainstem, cerebellum).

RESULTS:

Clinical observations: The author reported that untreated and antidote control groups appeared normal throughout the study and no clinical observations for these groups were presented. After the first treatment, pharmacologic signs (e.g., loss of equilibrium, decreased activity, diarrhea, and locomotor ataxia) were observed in 14of the 20 birds treated with the test material. These signs were observed on the day of dosing and cleared within five days. One additional animal showed pharmacologic signs (ataxia and torticollis) on the 15th day after the second treatment but appeared normal throughout the remainder of the study. Eight of the ten positive control animals showed signs of intoxication (e.g., locomotor ataxia, decreased activity, and diarrhea). Ataxia was observed beginning on day 12 and persisted in 3 birds until sacrifice. Two animals in the positive control group died during the study.

Mean body weights/food consumption: All but 3 animals exhibited weight loss during the study; thus, the mean body weights in all groups were depressed. Initial body weights for the negative controls were not reported. Food consumption varied widely. Disulfoton-treated animals had consistently lower food consumption rates than any of the other groups.

Pathology: Similar gross pathologic lesions were seen in all groups. Microscopic changes which the author considered "background lesions characteristic of aged laying hens" were observed in all groups. These changes included nerve fiber fragmentation and vacuolation, minimal to mild macrophage accumulation, and axonal swelling. Only one disulfoton-treated hen showed lesions more severe than background; however, this hen was diagnosed as having marked inflammation resulting from an infectious disease. Positive control hens had "more severe axonal" degeneration with moderate to marked macrophage accumulation and demyelination in the brain and spinal cord. However, the tabular results did not descibe the grading system used.

DISCUSSION:

Although pharmacologic signs (including locomotor ataxia) were observed in disulfoton-treated bird, these signs were considered to be a result of acute toxicity since they appeared on the day of dosing and/or did not persist. Positive control animals, on the other hand, showed clinical signs (e.g., delayed and persistent ataxia) characteristic of acute delayed neurotoxicity. Histopathology results in the test group were

comparable with concurrent untreated and antidote controls and according to the author comparable with background lesions observed in aged laying hens. Several shortcomings of the study were noted: healthy, young adult hens (8-14 months old) are required for an acute delayed neurotoxicity study, "retired" laying hens were used; forced motor activity outside the cage was not indicated; only 5 birds were used for the untreated control group, while a minimum of 6 birds are required; the derivation and the significance of the mean lesion grades were not described; and the significance of the mean lesion grades were not described; and the significance of the degeneration in the digestion chamber was not discussed.

Contradicting results were reported. For example, body weight in hen No. 2 in the positive control group increased by 80 g from 10/15/82 to 10/19/82 while food consumption was only 57 g. Between 11/5/82 and 11/9/82, hen No. 2 lost 87 g in body weight while consuming 367g and gained 104 g in body weight between 11/9/82 and 11/12/82 while consuming 288 g.

CONCLUSIONS:

Disulfoton does not produce acute delayed neurotoxicity in aged hens when given in two oral doses of 30 mg/kg body weight at 21-day intervals.

CORE CLASSIFICATION:

Supplementary Data.

# Demyelination Study on Hens

Fiche/Master ID 00057265

Taylor, R.E. (1965) Letter sent to Chemagro Corporation dated January 5, 1965: Report on Demyelination studies on hens: Report No. 15107. (Unpublished study received Mar. 24, 1965 under 6F0478; prepared by Harris Laboratories, Inc., submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:090534-C)

Test Chemical:

Di-Syston, purity unspecified

Experimental Protocol:

Twenty-four adult, high-laying strain, Leghorn hens were divided into four groups of six birds per group. Three different dose levels of Di-Syston were used; 2, 10, and 25 ppm by incorporation into the diet. One group served as a control. Poultry feed used was a standard composition layer mash formulation.

Birds were maintained on above treated feed for 30 days, after which the treated feed was replaced with untreated feed for all groups and feeding continued an additional 30 days. The birds were observed daily during the 60 day test period, for any evidence of leg weakness. At the end of the 30 day treatment period, three birds from each group were sacrificed and nervous tissues removed for histopathological examination. Nervous tissues examined included the spinal cord (cervical, lumbar sacral), sciatic nerve (longitudinal and cross section), and brain. Tissues were fixed in Fleming's solutions and stained with Osmic Acid Stain.

Results:

The author stated that "no abnormal symptoms were observed in any of the birds during the test period. Birds consumed feed and water normally at all times." Data on feed consumption are presented in the following table.

| Treatment Group | Average Feed Consumption per Bird per Day (gm) First 30 Days | Second 30 days |
|---|---|---|
| Control | 72.4 | 83.6 |
| 2 ppm Di-Syston | 71.3 | 82.4 |
| 10 ppm Di-Syston | 73.1 | 80.4 |
| 25 ppm Di-Syston | 72.7 | 84.6 |

The author further stated that no evidence of demyelination was observed in any of the tissues examined histopathologically.

Conclusion:

The author concluded that "Di-Syston did not cause demyelination in hens under the conditions of this experiment." However, it is our opinion that the lack of experimental details, inadequacy of the limited data provided and other deficiencies in the execution of this study would make it difficult to reach such a conclusion.

Core Classification:

Core-supplementary data.

003358

Acute Oral Toxicity to Farm Livestock

Fiche/Master ID 00013487

McCarty, R.T.; Haufler, M.; Osborn, M.G.; et al. (1969) Oral toxicity of four organophosphate insecticides to farm livestock. American Journal of Veterinary Research 30 (7): 1149-1153. (Report No. 25425; also 'In' unpublished submission received May 3, 1971 under 1F1[illegible]6; submitted by Chemagro Corp., Kansas City, Mo.; CDL:09[illegible]61-k)

Test Chemical:

Di-Syston emulsifiable concentrate (65.7%)

Experimental Protocol:

The nontoxic dose of Phorate was determined in newborn calves, yearling cattle, sheep, and goats. The maximal nontoxic dose was considered to be that dose that did not cause visible reaction in 20 animals of each of the various livestock classes except newborn calves where ten calves were considered sufficient to establish the maximal nontoxic dose for this group. Beside observing the reactions, in the animals, blood samples were drawn from each of the test animals before 6 hours after treatment and at periodic intervals thereafter to determine the activity of cholinesterase. The test chemical was given in a gelatin capsule as a single dose Observation of the treated animals were made at regular intervals for 30 days. Calves used were either Holstein-Friesian or Holstein-Friesian X Hereford crosses and weighed 30 to 50 kg each. Goats were Angora and weighed 15 to 25 kg each. The yearling cattle were Herefords or Hereford-Aberdeen Angus crosses and weighed 125 to 175 kg each.

Results:

The results obtained in this investigation are presented in Table 1.

Table 1: Oral Toxicity of Di-Syston in Farm Livestock

| Class of Livestock | No. of Animals | Dose mg/kg | % ChE activity | Remarks |
|---|---|---|---|---|
| Newborn calves | 1 | 1.0 | 14 | Poisoned, treated and recovered |
| | 8 | 0.5 | 28 | 6 NIE; 2 poisoned treated and recovered |
| | 10 | 0.25 | 59 | NIE |
| Yearling cattle | 1 | 2.5 | 32 | Poisoned, treated and recovered |
| | 4 | 1.0 | 44 | 3 NIE; 1 poisoned, treated and recovered |
| | 20 | 0.5 | 22 | NIE |
| Sheep | 3 | 5.0 | - | 3 poisoned, treated and died |
| | 4 | 2.5 | 43 | 2 NIE; 1 poisoned, treated and recovered; 1 poisoned, treated and died |
| | 22 | 1.0 | 60 | NIE |
| Goats | 3 | 2.5 | 29 | 1 NIE; 2 poisoned, treated and recovered |
| | 20 | 1.0 | 53 | NIE |

NIE - no ill effect
ChE - cholinesterase

According to the author signs of toxicosis in each class of livestock were those usually associated with the toxicosis caused by anti-cholinesterase compounds. The insecticide had marked anti-cholinesterase activity when administered in sufficient dose.

The toxic effects were progressive in severity and dependant on dosage and susceptibility of the test animal to the compound. Generally, nervousness was the first manifestation;

this was followed by the appearance of a slight frothy salivary accumulation on the lips. As toxicosis progressed, muscular trembling and excessive salivation with drooling were observed; these were accompanied by incoordination. In calves at this stage of toxicosis, protrusion of the tongue, abdominal cramps, and diarrhea were observed. The stools frequently had spots of blood on the surface. As toxicosis progressed further, ataxia became evident, and the test animal was unable to stand; uncontrolled muscular trembling was accompanied by convulsive spasms. Exhaustion occurred, and then death. At necropsy, the only gross pathologic change consistently observed was congestion of the lungs.

Conclusions:

The author concluded that the signs of poisoning and necropsy results were similar to those caused by many other organophosph insecticides. Newborn animals are more sensitive to Di-Syston than older animals of the same species.

The maximal nontoxic (NOEL) dose for each species is given in Table 2.

Table 2: Maximal Nontoxic (Nonlethal) Oral Dose of Di-Syston in 4 Classes of Livestock

| Classes of Livestock | Dose Level (mg/kg) | % of ChE inhibition |
|---|---|---|
| Newborn calves | 0.25 | 41 |
| Yearling cattle | 0.50 | 78 |
| Sheep | 1.00 | 40 |
| Goats | 1.00 | 47 |

Core Classification:

Not applicable, but the data are considered of supplementary nature.

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Experimental protocol and tables were taken from the original report.

Subacute Inhalation Toxicity Study on Female Rats

Fiche/Master ID 00087935

Dubois, K.P.: Kinoshita, F.K. (1971) Effect of Repeated Inhalation Exposure of Female Rats to Di-Syston: Submitter 30571, (Unpublished study received Nov. 30, 1971 under 3125-119; prepared by University of Chicago, Toxicity Laboratory, submitted by Mobay Chemical Corp., Kansas City, Mo.: CDL:10059-A)

Test Chemical:

Di-Syston, Batch No. 0050320, with unspecified purity.

Experimental Protocol:

According to the authors, young adult female Holtzman rats (225 to 250 gm) were used for these measurements. Groups of six rats were exposed to Di-Syston at concentrations of 0.14, 0.35, or 0.75 ug/liter of air for one hour per day for 5 or 10 days. The test material was dissolved in ethanol and the concentration was adjusted so that dispersing 2 ml/minute into a 200 liter dynamic flow gassing chamber gave the desired airborn concentrations. A standard glass Vaponephrin nebulizer was used to disperse the solution. The solution was dispersed at 3 lbs. of air pressure. After 5 days of exposure, half of the animals in each group were sacrificed after the tenth exposure. Cholinesterase activity was measured in brain, submaxillary glands and serum. Cholinesterase measurements were made by the manometric method of DuBois and Mangun. (Proc. Soc. Exper. and Med., 64, 137 (1947). No more experimental details were provided.

Results:

The data were presented as summary tables. The data indicated that the cholinesterase activity was not affected in any of the issues examined, at all dose levels.

Conclusions:

Airborn concentration of up to and including 0.75 ug/l was tolerated by female rats without any toxic signs.

Core Classification:

Core-supplementary data.

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003958

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Fiche Master ID 00069348

Bombinski, T.J.; Dubois, K.P. (1957) The Subacute Toxicity of Di-Syston to Rats: Report No. 1767. (Unpublished study received Nov. 20, 1957 under 3125-58; prepared by Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Corp., Kansas City, Mo.; CDL:100153-C).

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003958

Fiche Master ID 00000000

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Fiche Master ID 00071873

Crawford, C.R.; Anderson, R.H. (1973) The Acute Oral Toxicity of Several Di-Syston Metabolites to Female and Male Rats: Report No. 39687. (Unpublished study received December 15, 1976 under 3125-58; submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:095640-G)

Fiche Master ID 00069349

Doull, J. (1957) The Acute Inhalation Toxicity of Di-Syston to Rats and Mice: Report No. 1802. (Unpublished study received November 20, 1957 under 3125-58; prepared by Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:100153-D).

Fiche Master ID 00043213

DuBois, K.P. (1957) The Dermal Toxicity of Di-Syston to Rats: Report No. 2063. (Unpublished study received January 23, 1958 under unknown admin. no.; prepared by Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:109216-8)

Fiche Master ID 00089396

DuBois, K.P.; Doull, J.; Vaughn, G. (1958) The Effects of Diets containing Di-Syston on Rats: Submitted 2099. (Unpublished study received August 15, 1960 under PP0244; prepared in cooperation with Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:090271-H)



003958

Fiche Master ID 00087935

DuBois, K.P.; Kinoshita, F.K. (1971) Effect of Repeated Inhalation Exposure of Female Rats to Di-Syston: Submitted 30571, (Unpublished study received November 30, 1971 under 3125-119; prepared by University of Chicago, Toxicity Laboratory, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:10059-A)

Fiche Master ID 00089399

DuBois, K.P.; Vaughan, G.; Deininger, E.; et al. (1958) Determination of a Safe Dietary Level of Di-Syston for Dogs: Submitted 2573. (Unpublished study received August 15, 1960 under PP0244; prepared in cooperation with Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:090271-K)

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003958

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Fiche Master ID 00000000

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Fiche Master ID 00000000

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Fiche Master ID 00073348

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003958

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Thyssen, J. and Mohr, U. Disulfoton (S 276), the active ingredient of DI-SYSTON, subacute inhalation study on rats. An unpublished report (Bayer No. 9065, Mobay ACD No. 69361) prepared by Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, West Germany, for Mobay Chemical Corporation. April 1, 1980.

Subacute Inhalation Study in Rats

Fiche Master ID 000000000

Thyssen, J. and Mohr, U. (1980). Disulfoton (S 276), the active ingredient of ®DI-SYSTON, subacute inhalation study on rats. An unpublished report (Bayer No. 9065, Mobay ACD No. 69361) prepared by Bayer AG, Instute of Toxicology, Wuppertal-Elberfeld, West Germany, for Mobay Chemical Corporation. April 1, 1980.

Test Chemical:

Disulfoton 94.4%.

Experimental Protocol:

Toxicity experiments described below encompass two studies (Study I and II). Both studies were performed using Wistar TNO/W rats; the objectives of Study II was to determine a NOEL, which could not be determined in Study I, and to reproduce ~~the~~ the results observed in female rats of study I.

Study I (No. S 276/002):

1. Three groups of albino rats, each consisted of 10 animals/sex, with body weight range of 170-220 g at the beginning of the study, were exposed for 6 hours/day, 5 days/week, for three weeks to a nominal concentration of 0.5, 2.5, and 12.5 mg disulfoton/$m^3$. The test compound was "formulated in an ethanol/Lutrol mixture (1:1)." A fourth group of 10 animals/sex was similarly exposed to the solvent only at a concentration of 20 m/$m^3$.

2. The test compound was "dynamically nebulized" and animals were exposed in such a way that "no skin contact with the aerosols" occurred. The concentration of disulfoton in the exposure chamber was determined by gas chromatography "10 duplicate determinations." Actual concentrations were 0.1, 0.5, and 3.7 mg/$m^3$, corresponding to the nominal concentrations of 0.5, 2.5 and 12.5 mg/$m^3$, respectively. Particle size distribution was determined only once using cascade impactor; 73.9 to 90.8 percent of the particles were 1.5 u or less, and 96.0 to 99.7 percent were 3.0 u or less in size.

3. During the study animals were housed in Type III Makrolon cages (5 rats/cage) and were kept at approximately 21°C and 12 hours light/dark cycles.

4. Animals were observed daily for mortality and behavioral changes. Body weights were determined before exposure and weekly thereafter.

5. Twenty-four hours after the final exposure, 5 animals/sex/group were anesthetized by ether and blood was obtained by cardiac puncture for hematology and clinical chemistry tests. Hematology parameters studied included hematocrit, hemoglobin, mean corpuscular volume, erythrocyte and thromocyte counts, and total and differential leukocytes counts. Clinical chemistry parameters included SGOT, SGPT, alkaline phosphatase, plasma urea, and blood glucose levels.

6. Urine was collected from 5 animals/sex/group for 16 hours during the third week of exposure, and was analyzed for glucose, hemaglobin, albumin, urobilinogen and pH, and was examined microscopically.

7. Erythrocyte - and plasma-cholinesterase activity was determined before exposure and weekly thereafter on blood samples obtained from the retroorbital plexus using 5 animals/sex/group. Brain cholinesterase activity was determined at the end of the study using 5 animals/sex/group.

8. Twenty-four hours after the last exposure, animals were anesthetized by ether, exsanguinated, and gross pathology was performed. Absolute and relative organ weights were determined for the following organs: thyroid, heart, lungs, liver, spleen, kidneys, adrenals and testes/ovaries.

9. Histopathologic examination was performed on tissues from 5 animals/sex/group. These tissues included: heart, lungs, liver, kidneys, spleen, thyroid, adrenal, testes, esophagus, stomach, ovaries, eyes, bronchial lymph nodes, trachea, larynx, head, and bone marrow.

10. Statistical analysis was performed using the non-parametric Wilcoxin method to compare the means.

Study II (No. S 276/003):

1. In this study a group of 10 rats/sex, and another group of 20 female rats were exposed to disulfoton at nominal concentration of 0.1 and 12.5 mg/$m^3$, respectively, for 6 hours/day, 5 days/week for 3 weeks. Analytical concentrations of the test compound in the exposure chamber were 0.02 and 3.1 mg/$m^3$, respectively. Another control group of 10 rats/sex was similarly exposed to 20 ml/$m^3$ of the solvent (ethanol:Lutrol mixture 1:1).

2. Housing, feeding, and exposure method of animals, as well as particle size determination, observations, hematologic and clinical chemistry parameters, urinalyses, gross necropsy, organ weights, and histopathologic examination were the same as in Study I. About 92 ot 95 percent of the particles were 1.5 u or less in size.

3. Plasma-, erythrocyte-, and brain-cholinesterase activities were determined in animals that were exposed to 0.02 mg/$m^3$ only.

RESULTS:

Study I

Observations and Mortality: Animals in the highest dose group (3.7 mg/$m^3$) had muscular tremors, convulsions, increased salivation and "difficult breathing" during the first week (females), and at the end of the first week (males). Rats exposed to the intermediate dose level (0.5 mg/$m^3$) were lethargic; this effect was manifested on the second (females), and third (males) weeks of exposure. Both males and female animals in the lowest dose group were lethargic for "a brief time" after exposure during the third week of the study.

Five female rats from the highest dose group died during the study: one after each of the third, fourth, and 12th exposure, and two following the 10th exposure.

Body Weights: Body weights of male and female rats exposed to 0.1 and 0.5 mg/$m^3$ were comparable to that of the control group throughout the study. Animals exposed to 3.7 mg/$m^3$ of the test compound had lower body weights than the control group throughout the study (Table 1).

Hematology: No significant difference in hematologic parameters studies was found between the control and exposed anmimals.

TABLE 1. Mean Body Weights of Rats Exposed to Sulfoton for 3 Weeks

| Group | Sex | Mean Body Weights (g) Week 0 | Week 1 | Week 2 | Week 3 |
|---|---|---|---|---|---|
| Control | M | 199 | 196 | 202 | 207 |
| 3.7 mg/m$^3$ | M | 196 | 192 | 190 | 192 |
| Control | M | 175 | 173 | 173 | 173 |
| 3.7 mg/m$^3$ | M | 171 | 153 | 154 | 165 |

Clinical Chemistry: No significant change was observed in the clinical chemistry parameters studies except alkaline phosphatase which was increased in all exposed female animals as compared to the control. Females exposed to 0.1, 0.5, and 3.7 mg/m$^3$ of Disulfoton showed an increase in alkaline phosphatase averaging 9.1, 16.8, and 36.0 percent of the control value, respectively. However, examination of the individual animal data showed that clinical chemistry values in the highest concentration group were reported for one animal.

Urinalyses: Urinalyses showed no test compound-related effect.

Cholinesterase Activity: Both male and female animals exposed to 3.7 mg/m$^3$ of disulfoton showed a decrease in plasma-, erythrocyte-, and brain-cholinesterase activity. In addition, females exposed to 0.5 mg/m$^3$ showed a decrease in plasma-, and brain-cholinesterase activity. Plasma cholinesterase activity was also decreased in females exposed to 0.1 mg/m$^3$ (Table 2). As shown in Table 2 female animals were more sensitive to the inhibitory effect of Disulfoton on cholinesterase activity than males.

TABLE 2. Percent Decrease in Cholinesterase Activity of Exposed Animals as Compared to Pre-exposure Values*

| Cholinesterase Activity in | Dose Level mg/m$^3$ | Sex | Percent Decrease at Day 5 | 10 | 15 |
|---|---|---|---|---|---|
| Plasma | 3.7 | M | 67.3 | 80.0 | 70.9 |
| | 0.1 | F | 36.8 | 51.9 | 42.1 |
| | 0.5 | F | 47.0 | 51.7 | 44.4 |
| | 3.7 | F | 93.4 | 96.7 | 92.9 |
| Erythrocyte | 3.7 | M | 18.2 | 29.2 | 27.7 |
| | 3.7 | F | 28.3 | 32.0 | 39.7 |
| Brain | 3.7 | M | - | - | 47.5 |
| | 0.5 | F | - | - | 30.1 |
| | 3.7 | F | - | - | 57.7 |

*Changes in brain cholinesterase activity as compared to that of the control.

Gross Pathology: Female animals that died during the study showed mottled, distended, lungs; pale discoloration of the kidneys, bloated gastrointestinal tract; and ulcer-like foci in gastric mucosa. Animals sacrificed at the end of the study did not show test compound-related gross changes.

Organ Weights: No statistically significant compound-related effect was observed in the absolute or relative organ weights. An increase in the absolute adrenal gland weights averaging 14 percent of the control value was reported in the females exposed to 3.7 mg/m$^3$ of the test compound. This increases was ascribed by the authors to the test compound. However, because of the increase was not dose-related and the mean adrenal gland weights at the highest dose level were calculated using data from 5 animals only (versus 10 at other dose levels), it is the opinion of this reviewer that this effect was not test compound-related.

Histopathology: An increase in the round cells, perbronchial round cells, and perivascular round cells infiltrates was observed in male and female animals of all groups, and particularly in those exposed to 0.5 and 3.7 $m/m^3$ of the test material. Female animals exposed to 0.5 and 3.7 $mg/m^3$ of the test compound showed "reactive bone marrow changes in the forma of eosinophilia, toxic granulation, and nuclear abnormalities as well as increased plasmacytes." It should be noted that tissues from only one female animal of the high dose group were examined.

Study II

Observations and Mortality: Female rats exposed to 3.1 $mgm^3$ of the test compound showed signs of "inhibition of ChE activity" similar to those observed in Study I. One animal from the same group died after the 8th exposure and 2 more died after the 15th exposure.

Body Weights: Mean body weights of female rats exposed to the 3.1 $mg/m^3$ concentration were less than their initial body weights but not significantly different from the control.

Hematology: Except for a decrease in lymphocytes, averaging 22 percent of the control value, and an increase in polymorphmuclear leukocytes, averaing 225 percent of the control value in females at the 3.1 $mg/m^3$ level, no other significant change in hematology parameters was observed.

Clinical Chemistry: An increase in alkaline phosphatase level in female rats exposed to the 3.1 $mg/m^3$ level averaging 19.2 percent of the control value was observed. This increase, however, was not statistically significant. No changes were observed in other clinical chemistry parameters studied.

Urinalysis: No test compound-related effect was observed.

Cholinesterase Activity: The decrease in plasma-, and erythrocyte-cholinesterase activity in females exposed to 0.02 $mg/m^3$ was evident especially at the 5-day test period (Table 3). The decrease in plasma cholinesterase activity observed at day 5 in exposed females averaged 53.5 percent, control females showed a similar decrease averaging 26.8 percent of the pre-exposure value.

Gross Pathology: Female animals that died during the study showed distended lungs with dark discoloration. One animal

showed erythematous gastrointestinal tract. Sacrificed rats did not show any change that could be attributed to the test compound.

TABLE 3. Percent Change in Cholinesterase Activity in Females Exposed to the Test Compound at 0.02 mg/m$^3$ Level as Compared to Pre-exposure Values*

| Cholinesterase Activity in | Sex | Percent Change at Day 5 | 10 | 15 |
|---|---|---|---|---|
| Plasma | M | -27.6 | -20.7 | -6.9 |
| | F | -53.5 | -14.9 | +7.9 |
| Erythrocyte | M | -8.2 | -4.8 | -8.5 |
| | F | -26.5 | -16.8 | -19.7 |
| Brain | M | - | - | +10.9 |
| | F | - | - | +9.6 |

*Changes in brain cholinesterase activity as compared to that of the control.

Organ Weights: Increases in the absolute weights of lungs, liver, kidney and adrenals of females exposed to the 3.1 mg/m$^3$ level was observed. These increases averaged 9.1, 13.0, 6.6, and 9.3 percent of the control values, respectively. However, these increases were not statistically significant, and could be attributed to biological variations.

Histopathology: Inflammatory changes in the lungs and reactive bone marrow changes similar to those observed in Study I were noticed in females exposed to the 3.1 mg/m$^3$ level.

DISCUSSION:

In this report, experiments performed were divided into two studies: Study I in which groups of Wistar rats were exposed to 0.1, 0.5 or 3.7 mg/m$^3$ of Disulfoton, for 6 hours/day, 5 days/week, for three weeks. Because a NOEL could not be established, the study was repeated (Study II) with animals exposed to 0.02 mg/m$^3$, and 3.1 mg/m$^3$ of Disulfoton.

In both studies, animals exposed to the highest concentration shwoed a decrease in body weights as compared to the pre-exposure weights and control groups. The decrease in body weights was test-compound related. No data on food consumption were given.

Female rats were more susceptinle to the effects of Disulfoton than males. This was based on the following: 1) the decrease in body weights due to the test compound was more pronouced in females, 2) 50 percent adn 15 percent of females exposed to 3.7 and 3.1 mg/$m^3$, respectively, died during the study, 3) cholinesterase inhibition was more pronounced and occurred at lower dose levels in females than males, 4) reactive bone marrow changes were more severe in female animals.

The observed increase in alkaline phosphatase in females at all dose levels could be attributed to the changes in bone marrow since no histopathologic changes in the liver were observed. These effects on alkaline phosphatase and bone marrow were reproduced in Study II at the 3.1 mg/$m^3$ concentration. Furthermore, changes in differential leukocyte count reported in the second study were considered by the authors as a "first signs of response .... to the inflammation phenomena in the respiratory tract .... and to the bone marrow changes."

In the first study, plasma cholinesterase activity was decreased at all dose levels (0.1, 0.5, 3.7 mg/$m^3$) especially in female animals. Therefore, one group of animals in the second study was exposed to 0.02 mg/$m^3$ in order to established a NOEL. A decrease in plasma-cholinesterase activity averaging 27.6 percent and 53.5 percent was observed on day 5 of exposure in male and female animals, respectively. Similar decrease in erythrocyte-cholinesterase activity was also observed. Although these effects showed some reversal by the 15th day, 0.02 mg/$m^3$ cannot be considered a NOEL as concluded by the authors.

CONCLUSIONS:

Exposure of Wistar TNO/W74 rats to Disulfoton in concentrations of 0.02, 0.1, 0.5, 3.1, or 3.7 mg/$m^3$ for 6 hours/day, 5 days/week for three weeks resulted in a decrease in body weights at 3.1 and 3.7 mg/$m^3$, and mortality rates of 15 percent and 50 percent in females, respectively. Plasma cholinesterase activity was inhibited at all concentrations; erythrocyte- and brain-cholinesterase activity was also inhibited at the higher concentrations, especially in female animals. Alkaline

phosphatase levels were increased in female animals in all groups except for the group that was exposed to the lowest (0.02 mg/m$^3$) concentration. This effect could be attributed to the "reactive bone marrow changes" that were observed on histological examination of females; no liver lesions were observed. Inflammatory changes were also observed on histological examination of the lungs. The increase in polymorphnuclear leukocytes and the decrease in leukocytes in female animals were attributed to the changes in the lungs and bone marrow.

Since all concentrations of Dis[illegible]ton used, including the lowest concentration (0.02 mg/m[illegible] resulted in an inhibition of plasma cholinesterase activ[illegible] a NOEL could not be established from this study.

CORE CLASSIFICATION: Supplemen[illegible].

# Subacute Toxicity of Di-Syston to Rats

Fiche/Master ID 00069348

Bombinski, T.J.; DuBois, K.P. (1957) The Subacute Toxicity of Di-Syston to Rats: Report No. 1767. (Unpublished study received Nov. 20, 1957 under 3125-58; prepared by Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Corp., Kansas City, Mo.; CDL:100153-C).

Test Chemical:

Technical Di-Syston with unspecified purity.

Experimental Protocol:

This study was designed to ascertain the effects of daily exposure to relatively high levels of the chemical. Six groups of five young adult Sprague-Dawley rats were given daily ip injections of Di-Syston solution in 10% ethanol in propylene glycol at concentrations of 0, 0.25, 0.5, 1.0, 1.2, or 1.5 mg/kg. The concentration was adjusted so that the animals were given an amount equivalent to 0.1% of their body weight daily for a period of 60 days. The animals were observed for mortality and changes in body weight. At termination, surviving animals were sacrificed for cholinesterase measurements. Cholinesterase determinations were performed on the brains and serum of rats at termination. Three animals from each of the groups received 0.25, 0.5, and 1 mg/kg/day were sacrificed and cholinesterase activity was determined in the brain and the serum. According to the author to obtain further information on the effect of daily dosing administration of Di-Syston on the cholinesterase activity of rat tissues another series of animals was given 0.25, 0.5, or 1 mg/kg of Di-Syston daily. At various intervals groups of three animals which had received each of the doses of the compound were sacrificed and cholinesterase measurements were performed on brain and serum. The animals were always sacrificed 24 hours after the last injection of Di-Syston. Another series of rats was given 0.25, 0.5, or 1.0 mg/kg of Di-Syston and sacrificed at various intervals for cholinesterase assays. No further experimental details were provided.

Results:

General observation and mortality: according to the author, animals in the lowest two groups (0.25 and 0.5 mg/kg) produced no mortality and appeared normal except for some

decrease in body weight gain of the second group. At 1.0 mg/kg, animals exhibited cholinergic symptoms (lacrimation, salivation, urination, and tremors) and body weight loss continued until 7-10 days after which animals recovered and the body weight was back to the original level by day 15, and continued to increase thereafter. Figure 1 shows the effect of daily administration of Di-Syston on the growth rate. One animal died in the 1.0 mg/kg group after 10 days. About 80% of the animals received 1.2 or 1.5 mg/kg/day died in the first 10 days and 20% died after 10 days and in less than a month. At the end of the experiment the percent mortality was 20% in the middle dose group and 100% at greater dose levels. No mortality occurred in the lowest two dose levels (Table 1).

Table 1: Mortality of Rats Given Daily Intraperitoneal Doses of Di-Syston

| Daily dose of Di-Syston (mg/kg) | Days after the First Injection | | | | Mortality (60 days) |
|---|---|---|---|---|---|
| | 0-5 | 5-10 | 10-30 | 30-60 | |
| 0 | 0 | 0 | 0 | 0 | 0/5 |
| 0.25 | 0 | 0 | 0 | 0 | 0/5 |
| 0.5 | 0 | 0 | 0 | 0 | 0/5 |
| 1.0 | 0 | 0 | 1 | 0 | 1/5 |
| 1.2 | 2 | 2 | 1 | | 5/5 |
| 1.5 | 2 | 2 | 1 | | 5/5 |

Cholinesterase activity: two graphical illustrations were presented in the report for the effect of the treatment on brain and plasma cholinesterase. The graphical illustrations indicated that the percent inhibition increased with the increase in the dose level, and progressed with time in the first week, then leveled off (Figure 2 and 3). Each point on the curve represents an average of the four assays on the tissues of three animals.

Table 2: The Effect of Daily Intraperitoneal Doses of Di-Syston on the Cholinesterase Activity of Rat Tissues

| Daily Dose of Di-Syston (mg/kg) | Period of Treatment (days) | Total Dose (mg/kg) | % Control Cholinesterase* Activity | |
|---|---|---|---|---|
| | | | Brain | Serum |
| 0.25 | 60 | 15 | 42.6 (41.7-44.4) | 62.5 (57.7-68.6) |
| 0.5 | 60 | 30 | 33.3 (33.2-34.1) | 52.7 (43.8-57.7) |
| 1.0 | 60 | 60 | 14.9 (14.0-15.7) | 19.5 (13.4-23.1) |

* Average of four assays; numbers in parenthesis are the ranges of the four assays.

Discussion:

This study was designed primarily to obtain information on the extent to which repeated exposure to Di-Syston at frequent intervals result in subacute poisoning. However, in this study the test chemical was administered by intraperitoneal injection. The route of administration is not considered a normal way of exposure to pesticides.

The authors did not specify the sex of the animals used in this study. Toxicity of most organophosphorous insecticides is sex dependant. The investigators used 5 animals per group and did not specify the method of cholinesterase determination. No gross autopsy or histopathological examinations were performed. Limited parameters were examined. The data were presented as summary tables, and no raw data were available to ascertain the accuracy of these tables.

Conclusion:

The NOEL for plasma and brain cholinesterase inhibition could not be established. However, the animals tolerated a daily dose of Di-Syston up to and including 0.5 mg/kg for 60 days without mortality.

Core Classification:

Supplementary information.

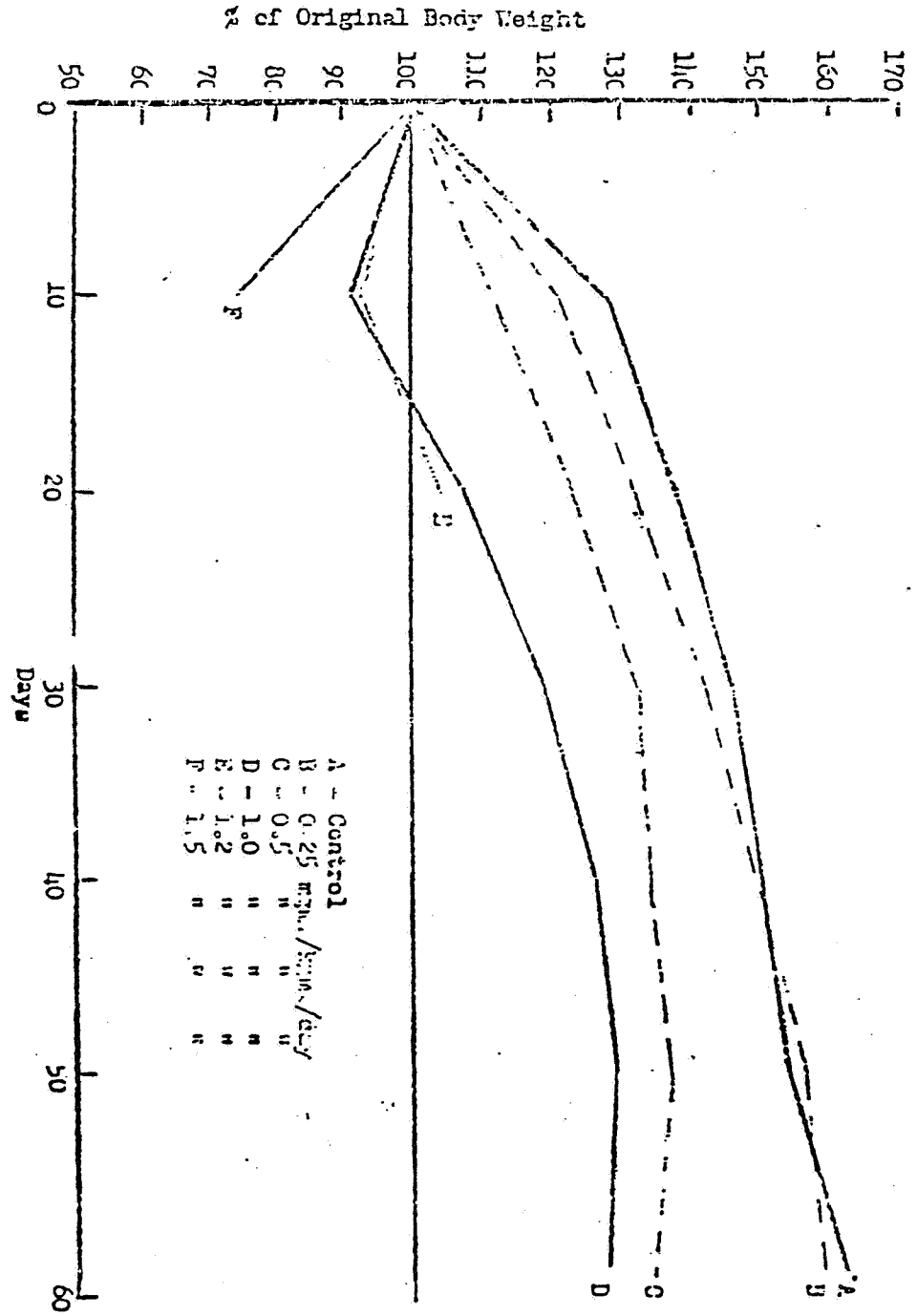



Figure 1

The Effect of Daily Intraperitoneal Administration of DL-Syston on the Growth Rate of Female Rats

Figure 2. Rate of Decrease of Brain Cholinesterase Activity After Repeated Daily Doses of Di-Syston

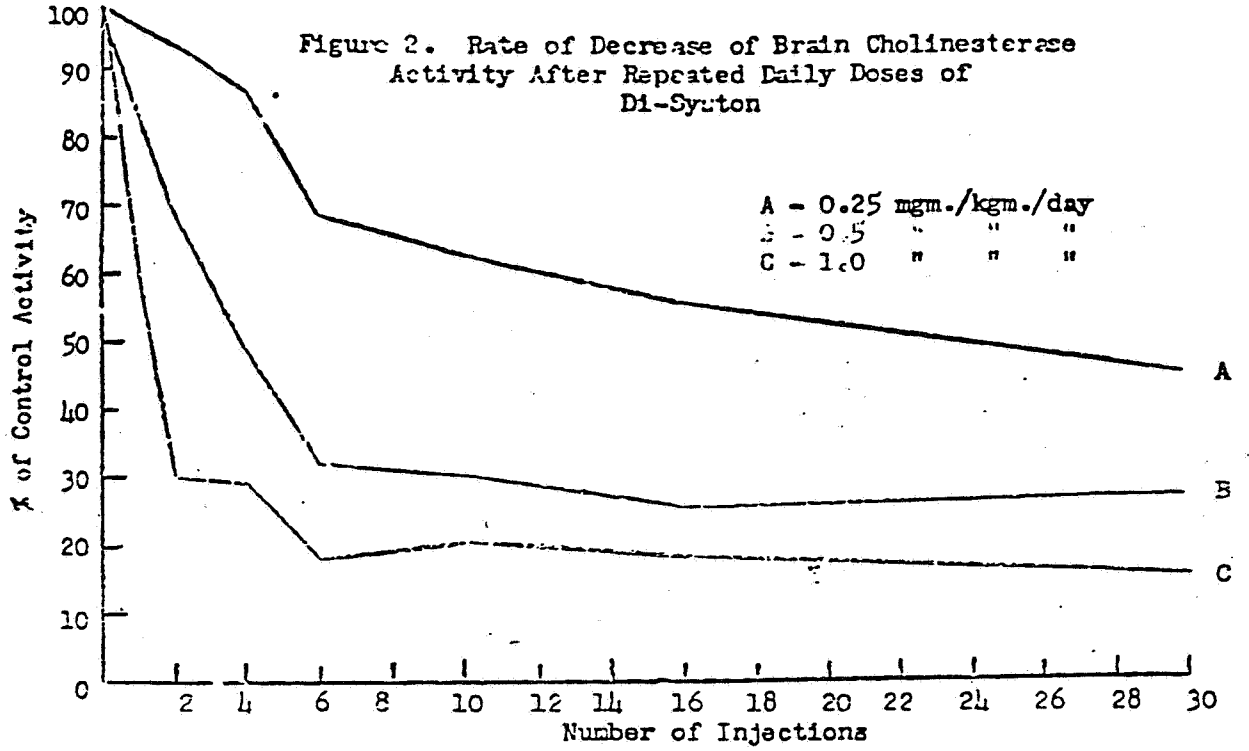
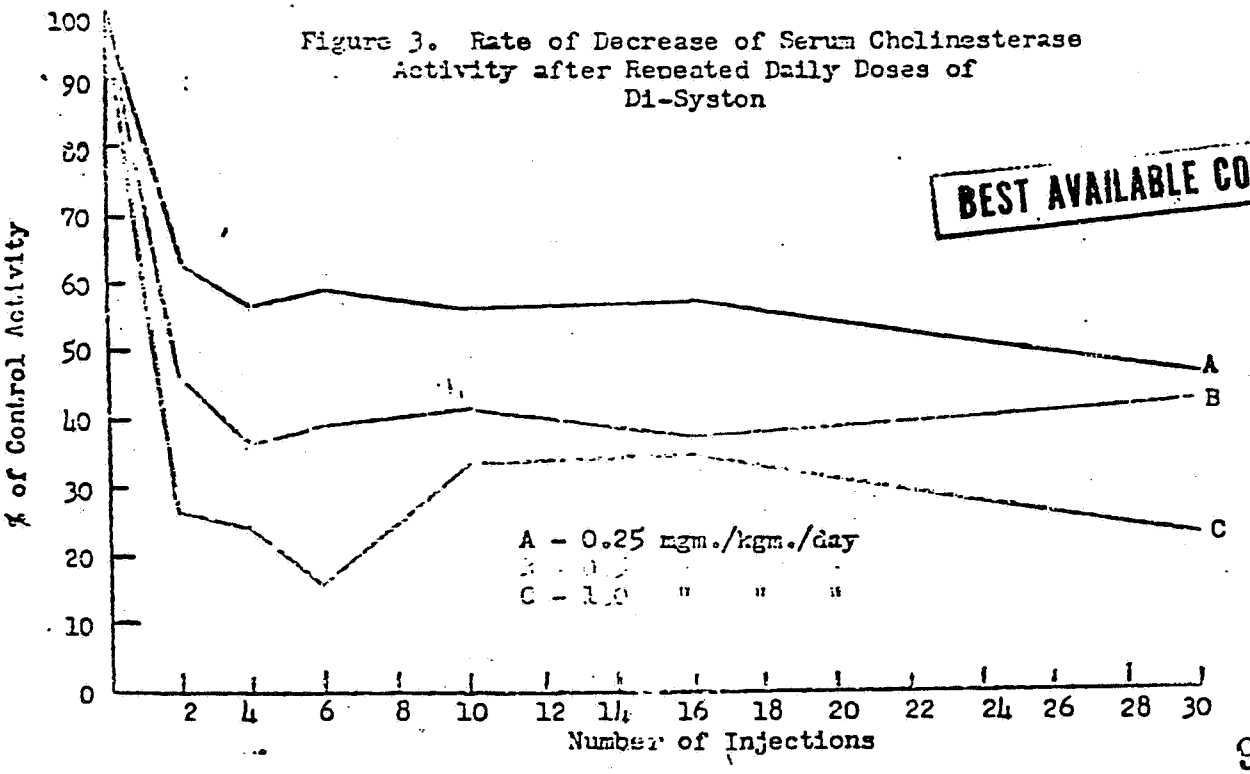



Figure 3. Rate of Decrease of Serum Cholinesterase Activity after Repeated Daily Doses of Di-Syston





003958

# The Effect of Repeated Dermal Exposure of Di-Syston and Its Metabolites on Serum Cholinesterase

Fiche/Master ID 00073344

Roensch, F. (1968) The Effect of Repeated Dermal Exposure of Di-Syston, Systox, Meta-systox-R and/or Their Metabolites on Serum Cholinesterase in the Rat: Report No. 21923. (Unpublished study received Dec. 15, 1976 under 3125-58; submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:095640-8)

Test Chemical:

Di-Syston technical, Di-Syston sulfoxide, and Di-Syston sulfon.

Experimental Protocol:

According to the author "twenty adult female Sprague-Dawley rats were used in this experiment. Four rats were in each of the treatment groups. The backs of the rats were closely clipped with an electric clipper. Eight rats remained untreated and served as controls. Each treated rat was housed in a separate stainless steel cage.

The insecticide and metabolites used in this experiment were: Di-Syston sulfoxide, Di-Syston sulfone, and Di-Syston technical. A 60% sucrose solution was used as the carrier in order to simulate plant sap and still obtain a product which would adhere to clipped back of the rat. The sucrose and water were heated until all the sucrose dissolved. The solution was cooled and a 100 ppm solution of each chemical was prepared. One ml of eah solution was placed on the shaved back of each rat for 5 consecutive days. Thus, each rat was dermally exposed to a total of 5 ml (0.5 mg active ingredient) of the solution.

The animals were anesthetized with ether 24 hours after the last treatment and a blood sample was obtained by cardiac puncture. Duplicate serum cholinesterase activity in each rat was determined by the Warburg manometric method (DuBois and Magnun, 1947).

Results:

The results of cholinesterase inhibition are presented in Table 1. The data did not indicate any major differences in the anticholinesterase potency between Di-Syston and its sulfoxide and sulfon metabolites.

Table 1: Effect of Dermal Exposure to Di-Syston and Its Metabolites on Serum Cholinesterase Activity in the Rat

| Di-Syston sulfoxide | | Di-Syston sulfone | | Di-Syston Tech. | |
|---|---|---|---|---|---|
| Rat No. | $CO_2$ ul 10-40 min. | Rat No. | $CO_2$ ul 10-40 min. | Rat No. | $CO_2$ ul 10-40 min. |
| 1-1 | 42.5 | 2-1 | 53.6 | 3-1 | 50.3 |
| 1-1 | 42.1 | 2-1 | 51.8 | 3-1 | 50.1 |
| 1-2 | 68.5 | 2-2 | 47.0 | 3-2 | 53.8 |
| 1-2 | 65.1 | 2-2 | 47.2 | 3-2 | 53.5 |
| 1-3 | 42.4 | 2-3 | 35.7 | 3-3 | 48.4 |
| 1-3 | 42.2 | 2-2 | 34.0 | 3-3 | 48.8 |
| 1-4 | 43.8 | 2-4 | 44.6 | 3-4 | 45.2 |
| 1-4 | 41.3 | 2-4 | 45.6 | 3-4 | 46.7 |
| Mean | 48.5 | Mean | 44.9 | Mean | 49.6 |
| Per cent inhibition | 6 | Per cent inhibition | 63.2 | Per cent inhibition | 59.5 |

Discussion and Conclusions:

Insufficient experimental details was provided. The limited data presented in this report indicated that Di-Syston and its two metabolites did not differ in their cholinesterase inhibiting potency. However, it should be emphasized that the rate of dermal uptake is very much dependant on the polarity of the applied test material. Since Di-Syston and its metabolites are different in their polarity, and thus rate of dermal uptake, the basis for this comparison is incorrect. Furthermore, only one concentration was applied and for a very short time.

Core Classification:

Supplementary data.

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Data reported in this review are only those that belong to Di-Syston.

003958

Subchronic Feeding Toxicity in the Dog

Fiche/Master ID 00089399

DuBois, K.P.; Vaughn, G.; Deininger, E.; et al. (1958) Determination of a Safe Dietary Level of Di-Syston for Dogs: Submitted 2573. (Unpublished study received Aug. 15, 1960 under PP0244; prepared in cooperation with Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:090271-K)

Test Chemical:

Di-Syston 25% Wettable Powder

Experimental Protocol:

According to the author, "three groups of adult mongrel dogs each of which contained one male and one female animal were employed for these studies. The dogs were housed in individual cages and were provided with water and dog food (Arcady Dog Chow) containing dietary levels of 1, 2 and 10 ppm. Each of the experimental groups was fed one of these diets for twelve weeks. Prior to initiating the Di-Syston feeding, all of the animals were fed a control diet containing no Di-Syston for four weeks during which time measurements of the plasma and erythrocyte cholinesterase activity were made on each animal weekly. The results of these studies were used to establish the base-line values for the cholinesterase determinations which were made weekly during the Di-Syston feeding. Following the completion of the 12-week Di-Syston feeding period the animals were returned to the control diet and the cholinesterase determinations continued to obtain information on the recovery rate of this enzyme in the blood.

The plasma and erythrocyte cholinesterase activity was determined using the method of DuBois and Mangun (1) and duplicate determinations were made on the blood of each animal using 200 mg. of plasma and 200 mg. of packed red blood cells for each determination.

During the Di-Syston feeding period the dogs were observed daily for evidence of toxic symptoms and were weighed at weekly intervals.

Results:

Cholinesterase activity: data on the control levels of cholinesterase activity for the plasma and red blood cells are presented in Table 1.

Table 1: Control Levels of Plasma and Erythrocyte Cholinesterase Activity in Male and Female Dogs

| Dose Group | Sex | Cholinesterase Activity* | |
|---|---|---|---|
| | | Plasma | Erythrocytes |
| 1 ppm | Male | 12.1 (11.7-12.5) | 12.9 (12.6-13.1) |
| | Female | 17.7 (17.5-17.8) | 19.9 (19.7-20.0) |
| 2 ppm | Male | 22.4 (22.3-22.5) | 16.2 (15.8-16.6) |
| | Female | 11.7 (11.3-12.1) | 11.7 (11.3-12.0) |
| 10 ppm | Male | 16.7 (16.0-17.3) | 11.1 (10.7-11.4) |
| | Female | 13.6 (13.0-14.0) | 14.7 (14.5-14.9) |

* Expressed as microliters of $CO_2$ evolved per 10 mins. per 50 mg. of plasma or packed erythrocytes. The values were obtained from at least four duplicate determinations made over a period of one month and represent the mean and the range of values in parentheses. The standard deviation of any value did not exceed more than 0.65 units from the mean.

In this study each dog served as its own control. No other controls were included. Data on cholinesterase inhibition were presented graphically. The graphical illustrations indicated that the inhibition of plasma and erythrocyte cholinesterase was progressive with the dose level and time. Dose level of 1 ppm caused a 10% or less inhibition in plasma and erythrocyte cholinesterase. In the middle dose group, the maximum inhibition level was attained after three weeks in the plasma, (50%) and after about 8 weeks in erythrocytes (30%). In the high dose group the maximum inhibition occurred at one week (over 80%) in the plasma and at three weeks (over 80%) in the erythrocytes. In all cases the activity of cholinesterase started to increase when the animals were placed on Di-Syston-free diets after the three months feeding experiment. The figures

presented above were estimated from the graphical illustration of the cholinesterase data as presented in the original report.

Effect on body weight gain: the data on body weight gain were presented graphically. According to the authors all the animals included in this study were weighed at weekly intervals during the feeding period. The author stated that "none of the dogs lost weight as a result of the Di-Syston feeding. There were no toxic symptoms or evidence of cholinergic stimulation in any of the Di-Syston fed dogs during the 12-week feeding period.

Conclusions:

The primary target for the effect of Di-Syston is the cholinesterase. A NOEL could be established at 1 ppm, and an LEL at 2 ppm.

Core Classification:

Supplementary Data. Only one animal per sex per dose group was used. Data were reported as an average per the male and female in each group, and presented graphically with no raw data. Very limited number of parameters were tested.

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1. DuBois, K.P. and Magnum, G. (1947). Proc. Soc. Exper. Biol. Med., 64, 137.

2. Some of the material included in this review were taken from the original report.

003958

Subchronic Feeding Toxicity in the Rat

Fiche/Master ID 00089396

DuBois, K.P.; Doull, J.; Vaughn, G. (1958) The effects of Diets Containing Di-Syston on Rats: Submitted 2099. (Unpublished study received Aug. 15, 1960 under PP0244; prepared in cooperation with Univ. of Chicago, Dept. of Pharmacology, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:090271-H)

Test Chemical:

Di-Syston 25% wettable powder.

Experimental Protocol:

Male and female Sprague-Dawley rats weighing 54-88 grams were used in this study. Five groups at least 13 males and 13 females were maintained on diets containing 0, 1, 2, 5 or 10 ppm for a period of 16 weeks. The diets were prepared on a weekly basis. The animals were housed individually and had constant access to food and water. Food consumption and body weights were measured daily in the first two weeks at two day intervals for the rest of the month and at weekly intervals thereafter.

At termination three males and three females of each group were sacrificed. According to the author the following tissues were removed, weighed, and prepared for histological examination: brain, lung, heart, spleen, stomach, kidney, adrenal gland, liver, urinary bladder, and submaxillary glands. Portions of the following tissues wee also prepared for similar examinations: small intestine, large intestine, skeletal muscle, gonads (testes weighed), spinal cord, portions of the sternum (for bone marrow observation) and a section of the left femur. All tissues were fixed in buffered formalin, imbeded, sectioned and stained with hematoxylin-eosin except for the sections of spinal cord which were stained with the Lillie modification of the Weil-Weigert stain to demonstrate the myelin sheaths.

According to the authors, cholinesterase activity of the serum, erythrocytes, brain, and maxillary gland at 8 weeks and then at termination. At least three animals of each sex were used from each dose group for this determination. Cholinesterase determination was performed in duplicate using the manometric method of DuBois and Mangun (Proc. Soc. Exper. Med. 1947. 64, 137).

Results

Food consumption and body weight: the results of the effect of Di-Syston food consumption and growth were presented graphically in this report. The treatment did not affect the food consumption or the rate of growth of both males and females.

Effect on tissue cholinesterase: the results of cholinesterase determination are presented in the original report in a summary form as the mean and the range for each group. According to the authors, animals in the highest dose group exhibited marked inhibition of cholinesterase of the brain, submaxillary gland and red blood cells. The serum cholinesterase of the females in this group was over 80% inhibited, but the serum cholinesterase of males in the same group was only 25% inhibited. The inhibition was consistently more pronounced in the tissues of males. There was no inhibition of the serum of male rats fed diets containing less than 10 ppm of Di-Syston. On the other hand, dietary levels of 1 ppm caused 15% inhibition of the red blood cell cholinesterase in female rats. According to the authors, the range of values of the erythrocyte cholinesterase activity for females fed 1 ppm overlaps the range of values for the control animals, and the inhibition after 16 weeks was no greater than that observed following 8 weeks of Di-Syston feeding, thus it appears that males and females would be able to tolerance a level of 1 ppm of Di-Syston in the diet. Male rats were able to tolerate diets containing up to 2 ppm of Di-Syston for 16 weeks without significant inhibition of the cholinesterase of the tissues with the exception of the red blood cells. Increasing the level to 5 ppm resulted in inhibition of the cholinesterase activity of almost all of the tissues in both males and females within the first 8 weeks of the feeding period.

Organ Weights: data on organ weights are presented in Table 3. The feeding of Di-Syston did not significantly affect organ weights.

Gross and histopathological examination: the examination was done on three males and three females of each dose group. One male of the 2 ppm group exhibited marked prostatic enlargements and numerous areas of focal necrosis in the surrounding area. Large amounts of hemosiderin were present in the macrophages in the speen of both the control and the treated animals. Peribronchial acummulation of lymphoid tissues was noted in the lungs of the control and treated animals. Vasculation of the cytoplasm of the hepatic cells was observed in the controls and the treated animals, but the incidence was significantly higher on the treated animals.

Table 1: Effect of Di-Syston in the Diet on the Tissue Cholinesterase Activity of Male Rats

| Dietary Level of Di-Syston | Weeks of Feeding | Tissue Cholinesterase Activity[a,b] | | | |
|---|---|---|---|---|---|
| | | Serum | Erythrocytes | Submaxillary Gland | Brain |
| 0 ppm (Controls) | 8 | 4.8 (4.5-5.1) | 8.7 (5.7-9.7) | 20.6 (20.0-20.9) | 92.0 (88.1-95.3) |
| | 16 | 5.2 (4.9-5.6) | 9.3 (8.5-10.0) | 23.5 (20.1-25.7) | 89.4 (88.9-92.3) |
| 1 ppm | 8 | 5.6 (5.0-5.8) | 8.4 (7.8-8.9) | 21.8 (21.0-24.1) | 92.9 (90.2-94.4) |
| | 16 | 5.8 (5.1-6.3) | 9.7 (7.8-10.2) | 20.3 (17.7-24.7) | 88.3 (82.3-93.4) |
| 2 ppm | 8 | 5.4 (4.8-5.9) | 7.7 (7.0-8.1) | 19.1 (17.7-19.9) | 78.8 (74.1-88.1) |
| | 16 | 5.2 (5.0-5.4) | 6.5 (5.9-7.1) | 23.5 (22.5-27.2) | 80.9 (76.5-85.4) |
| 5 ppm | 8 | 5.2 (4.5-5.8) | 5.6 (3.5-7.6) | 11.3 (8.8-14.0) | 61.9 (56.2-63.7) |
| | 16 | 5.7 (4.0-6.1) | 4.7 (4.2-5.1) | 14.4 (12.5-16.2) | 65.2 (64.5-66.0) |
| 10 ppm | 8 | 3.5 (3.3-3.6) | 3.2 (2.8-3.5) | 9.3 (8.7-9.9) | 55.5 (50.0-67.7) |
| | 16 | 3.7 (3.4-3.9) | 3.1 (2.8-3.3) | 11.2 (9.1-13.3) | 48.7 (45.9-51.4) |

[a] Values obtained from at least 3 animals. Range of values in parentheses.

[b] Cholinesterase activity in microliters of $CO_2$ produced/10 minutes/50 mg. of tissue (wet weight)

Table 2: Effect of Di-Syston in the Diet on the Tissue Cholinesterase Activity of Female Rats

| Dietary Level of Di-Syston | Weeks of Feeding | Tissue Cholinesterase Activity[a,b] | | | |
|---|---|---|---|---|---|
| | | Serum | Erythrocytes | Submaxillary Gland | Brain |
| 0 ppm (Controls) | 8 | 15.2 (13.6-16.8) | 12.5 (9.1-13.8) | 21.9 (18.2-24.8) | 90.6 (88.5-95.2) |
| | 16 | 18.7 (16.2-22.2) | 11.0 (10.7-11.2) | 23.5 (21.5-24.4) | 90.1 (87.5-94.4) |
| 1 ppm | 8 | 14.6 (12.8-16.8) | 10.5 (9.6-10.9) | 20.9 (18.9-23.0) | 88.9 (87.4-89.9) |
| | 16 | 17.6 (17.0-19.3) | 9.5 (7.8-11.0) | 21.9 (20.0-23.0) | 80.4 (79.3-82.4) |
| 2 ppm | 8 | 14.0 (11.7-16.3) | 5.1 (5.0-5.4) | 16.1 (12.4-18.8) | 73.4 (71.0-75.7) |
| | 16 | 16.5 (13.2-18.7) | 6.5 (6.3-6.9) | 18.3 (17.8-18.8) | 67.3 (62.7-72.4) |
| 5 ppm | 8 | 3.8 (3.7-3.9) | 2.1 (1.8-2.4) | 11.3 (8.2-13.4) | 55.5 (51.9-59.0) |
| | 16 | 2.4 (1.8-2.8) | 2.3 (2.1-3.9) | 8.2 (5.3-11.1) | 38.5 (36.5-40.5) |
| 10 ppm | 8 | 2.7 (2.4-2.9) | 2.1 (1.6-2.6) | 9.1 (8.2-10.7) | 31.3 (29.9-32.6) |
| | 16 | 2.4 (2.4-3.6) | 1.5 (1.4-1.7) | 7.4 (6.8-7.9) | 16.5 (14.1-18.8) |

[a] Values obtained from at least 3 animals. Range of values in parentheses.

[b] Cholinesterase activity in microliters of $CO_2$ produced/10 minutes/50 mg. of tissue (wet weight)

Table 3: Effect of Di-Syston on Organ Weights

| Organ | Dietary Concentration of Di-Syston | | | | |
|---|---|---|---|---|---|
| | Controls | 1 ppm | 2 ppm | 5 ppm | 10 ppm |
| Brain | 1.626* | 1.674 | 1.626 | | 1.787 |
| | (450) | (554) | | (472) | (538) |
| Heart | 1.053 | 0.975 | 1.023 | 1.084 | 0.949 |
| | (292) | (323) | (280) | (297) | (286) |
| Kidney(left) | 1.192 | 1.006 | 1.180 | 1.102 | 1.049 |
| | (330) | (333) | (323) | (320) | (316) |
| Lung | 1.690 | 1.436 | 1.720 | 1.741 | 1.628 |
| | (468) | (475) | (471) | (476) | (487) |
| Liver | 13.4 | 11.7 | 12.6 | 12.4 | 11.4 |
| | (3710) | (3870) | (3450) | (3400) | (3430) |
| Spleen | 0.606 | 0.557 | 0.658 | 0.646 | 0.592 |
| | (168) | (184) | (180) | (176) | (178) |
| Testis (1) | 1.740 | 1.710 | 1.748 | 1.711 | 1.702 |
| | (480) | (566) | (476) | (468) | (492) |
| Bladder (urinary) | 0.157 | 0.108 | 0.125 | 0.118 | 0.101 |
| | (43) | (36) | (34) | (32) | (30) |

* average weight of organs in grams obtained from at least 3 male and 3 female rats except for the testis weights where both testis from the 3 male rats were weighed. Number in parenthesis is the average organ weight in mg./100 g of body weight.

Conclusion

The primary target for the effect of Di-Syston is the cholinesterase. A NOEL can be established at 1 ppm and an LEL at 2 ppm. However, this "no observed effects level" should be considered with some reservation since some cholinesterase inhibition (15%) was observed in the red blood cells of the females of this group.

Core Classification:

Core supplementary data. A very limited number of parameters were investigated. The raw data were not submitted. The test material was not the technical.

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Some of the materials included in this report were taken from the original report.

003958

Two-Year Feeding and Oncogenicity Study in the Rat

Fiche/Master ID 00069966

Carpy, S.; Klotzsche, C.; Cerioli, A. (1975) Disulfoton: 2-Year Feeding Study in Rats: AGRO DCK CBK 1851/74; Report No. 47069. (Unpublished study received Dec. 15, 1976 under 3125-58; prepared by Sandoz, Ltd., Switzerland, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:095641-C)

Test Chemical:

Technical Di-Syston 95.7%.

Experimental Protocol:

Test Animals: 480 SPF Sprague-Dawley rats (in-house breeding) were divided in four groups, each group consisting of 60 (M) and 60 (F). One group was used as control (K) and the three other groups: (A), (B), and (C) were fed diet treated with Di-Syston at the rate of 0.5/5.0, 1.0 and 2.0 ppm respectively, for 104 weeks. All animals were caged individually.,

The rats were 4-5 weeks old at the beginning of the experiment.

Dosage: A 50% Ultracil premix was prepared from technical Di-Syston. The premix was added to the powdered standard rat diet (NAFAG No. 895) to give the following Di-Syston concentrations:

| Group | Dosage in ppm |
|---|---|
| K | 0.0 |
| A | 0.5/5.0 |
| B | 1.0 |
| C | 2.0 |

The lowest dietary level 0.5 ppm was increased to 5.0 ppm on the 81st week because the 1.0 ppm level showed no adverse effects. The animals treated with the 2 ppm dietary level were initially kept on 1.5 ppm dietary level the first 4-5 weeks of this experiment. The treated feed was prepared every two weeks.

Parameters Examined: The animals were observed daily. Body weight, food consumption and water intake of 15 animals per dose per sex were recorded weekly. Weight change, food and water consumption, efficiency of food utilization (EFU), and intake of test compound were computed weekly and bi-weekly.

Hematology, blood chemistry in plasma and urinalysis were carried out on 10 animals per dose group (5 males and 5 females).

Both hematology and clinical chemistry were done after 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 months of treatment. Urinalysis was done after 1, 3, 6, 12, 18, and 24 months of treatment.

Hematology studies included hematocrit (HCT), Hemoglobin (HGB), Red blood cell count (RBC), Absolute Indices: Mean cell volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCH ); Differential count: Lymphocytes (LYMPHO), Monocytes (MONO), eosinophils (EOSINO), basophils (BASO), neutrophil band (BAND.N.) and segments (SEGM.N) % WBC.

Clinical Chemistry Studies were performed on blood samples taken from animals after fasting overnight. Cholinesterase (ChE) activity was tested for plasma and red blood cells (Brain ChE activity and liver ChE were also measured at termination), also serum Glutamic-pyruvic transaminase (SGPT), Alkaline Phosphatase (ALCPHOS), Plasma glucose (GLUC), Plasma urea nitrogen (BUN), Total plasma cholesterol (CHOL).

Urinalysis - 5 males and 5 females were individually housed for 24 hours in special cages; the animals had access to water only. Urine was collected overnight and tested for the following: pH, specific gravity, protein, glucose, blood pigments, ketones, and microscopy of sedimentation.

Necropsy was done on 10 animals per sex per dose group after 104 weeks of dietary treatment; the remaining animals were also examined for tumors. Food was removed 24 hours before necropsy, and the animals were killed with Nembutal. Terminal body weight and weight of the following organs were recorded.

| | | |
|---|---|---|
| thyroid | thymus | ovaries |
| adrenals | heart | prostates |
| kidneys | lungs | uterus |
| spleen | seminal vesicles | brain |
| liver | testes | pituitary |

Organ to body weight ratios were calculated.

Histopathology was performed on the control group and the 5 ppm group. The following tissues from 5 animals per sex were examined:

| | |
|---|---|
| salivary gland | esophagus |
| mammary gland | stomach |
| skin | duodenum |
| mesenteric lymph nodes | small/large intestine |
| thyroid | urine bladder |
| adrenal | sciatic nerve |
| spleen | bone marrow (femur) |
| pancreas | skeletal muscle |
| liver | seminal vesicle |
| thymus | testes/ovaries |
| heart | prostate/uterus |
| lungs | eyes |
| trachea | optic nerve |
| tongue | brain |
| | pituitary |

These tissues were preserved in Bouin's solution or 4% formaldehyde.

Statistical Method: Student t-test was used to assess the inter-group differences; U-test was also at some point of the experiment. We noticed that Kastenbaum-test was also used.

Results:

Food Consumption and Total Di-Syston Intake

The average food consumption was similar in all groups, with 16.8-17.6 g/day/male and 13.0-13.7 g/day/female.

No differences were observed in the food efficiency between any treated group and the control animals during the study.

Diet was not analyzed, however, the average daily drug intake was calculated based on the weekly mean food intake/group.

| Nominal Dosage | | Calculated Daily Di-Syston Intake mg/kg/day | |
|---|---|---|---|
| ppm | mg/kg/day | Males | Females |
| 0.5/5.0* | 0.025/0.250 | 0.0215/0.1900 | 0.0267/0.1960* |
| 1.0 | 0.050 | 0.0456 | 0.0419 |
| 2.0 | 0.100 | 0.0893 | 0.1033 |

* On a time weighted basis, this dosage is equivalent to 1.5 ppm = 0.075 mg/kg/day.

Mortality

The total mortality of rats at the end of 104 weeks was as follows:

| Group | Dosage (ppm) | Mortality Ratio Males (%) | Females (%) |
|---|---|---|---|
| K | 0.0 | 33/60 (55) | 27/60 (45) |
| A | 0.5/5.0 | 36/60 (60) | 23/60 (38) |
| B | 1.0 | 36/60 (60) | 25/60 (42) |
| C | 2.0 | 45/60 (75) | 19/60 (32) |

The highest mortality rate observed was in males at the 2.0 ppm dose level. Change of the 0.5 ppm dosage to 5.0 ppm level for the last 23 weeks of the study did not substantially increase the mortality rate.

Body Weight

Di-Syston did not cause significant differences in body weight between control and treated animals at the dosages tested.

Hematology

All values were within the normal range; no time or test compound related changes were observed.

Clinical Chemistry

Blood glucose values were within the normal range for all animals. However, alternating increased (10-24%) and decreases (15-24%) were noted at 1 ppm and 2 ppm dosages as compared to the control group. These fluctuations persisted until week 91 in males at these two dose levels, and were noted in females until week 13.

BUN values were normal in females during the whole study. Males demonstrated an increase in control and in all treated animals after week 78, which may indicate that this change is related to age.

No significant difference was observed between treatment and control in the transaminase or alkaline phosphatase activity. Total blood protein and cholesterol were not measured until week 52, however no difference was observed between control and treatment groups.

Urinalysis

No significant difference was observed between control and treatment groups.

Cholinesterase

Statistically significant cholinesterase inhibition was noted in plasma (PChE), red blood cells (RBCChE), and brain (BChE) at 2.0 ppm and 5 ppm dosages in both males and females.

We calculated the percentage ChE inhibitions at termination based on ChE activities in the control animals, these percentages were as follows:

| | | | |
|---|---|---|---|
| 2 ppm dosage - | PChE | 14% ($p < .05$) M; | 22% ($p < .05$) F |
| | RBCChE | 9% ($p < .01$) M; | 13% ($p < .05$) F |
| | BChE | 9% M; | 17% ($p < .01$) F |
| 5 ppm dosage - | PChE | 20% ($p < .01$) M; | 40% ($p < .001$) F |
| | RBCChE | 18% ($p < .001$) M; | 27% ($p < .001$) F |
| | BChE | 25% ($p < .01$) M; | 36% ($p < .001$) F |

RBCChE inhibition at 2 ppm and 5 ppm dosages seemed to be lower at termination than during the study. At the 2 ppm dose level, females RBCChE inhibition was the greatest - 27% and 22% in weeks 39 and 91 respectively; male RCBChE inhibition was the greatest - 23% and 21% in weeks 64 and 26 respectively. Since the food was removed 24 hours before termination, it is likely that this contributed to the reduction of ChE inhibition data at termination.

Liver cholinesterase (LChE) activity was also investigated at termination. Calculations based on protein content in the liver homogenate showed that ChE activity increased in both males and females at 1 ppm dose level; at the 2 ppm and 5 ppm dietary levels males responded differently than females. The males demonstrated decreased LChE activity 5% and 23% at 5 ppm and 2 ppm dose levels respectively; the females demonstrated incrased LChE activity 20% and 30% at 2 ppm and 5 ppm dose levels respectively, the activation was dose-related.

These LChE activities demonstrated that Di-Syston induced hepatic enzyme activation in females at the dose levels investigated; this effect was also noted in males at 1 ppm level, however at higher Di-Syston levels this activation mechanism seemed to be inhibited. Failure of this mechanism in males at the higher dose levels may have been a factor in the higher mortality rate noted at 2 ppm where LChE inhibition was the greatest 23%.

The following table represents the activity and percent inhibition of PChE, RBCChE, and BChE at termination, as the mean of five animals/sex/group.

| ChE | Dosage in PPM | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | | 1.0 | | 2.0 | | 0.5/5.0 | |
| | M | F | M | F | M | F | M | F |
| PChE | | | | | | | | |
| Activity (U/ml) | 1.54 | 4.07 | 1.56 | 3.35 | 1.32* | 3.13* | 1.22** | 2.46** |
| % Inhibition | 0.0 | 0.0 | 0.0 | 7.7 | 14.0 | 22.0 | 20.0 | 39.6 |
| RBCChE | | | | | | | | |
| Activity (U/ml) | 3.44 | 3.45 | 3.37 | 3.23 | 3.21** | 2.99* | 2.82*** | 2.51** |
| % Inhibition | 0.0 | 0.0 | 5.0 | 6.4 | 9.3 | 13.3 | 18.3 | 27.1 |
| BChE | | | | | | | | |
| Activity (U/ml) | 24.89 | 25.99 | 23.75 | 23.24** | 22.61 | 21.53** | 18.74** | 16.66* |
| % Inhibition | 0.0 | 0.0 | 0.0 | 11.0** | 9.0 | 17.0 | 25.0 | 36.0 |

t-test: * = $p < 0.05$ ** = $p < 0.01$ *** = $p = 0.001$
% inhibition as compared to the control.

Females were 1.5 - 2.0 times more sensitive than males to PChE, RBCChE and BChE inhibition by Di-Syston.

RBCChE and BChE inhibition data reflected a NOEL of 1 ppm and an LEL of 2 ppm.

Necropsy and Histopathology

Mean organ weights of 10 animals/sex/group were reported. The tissues were then fixed in Bouin's or 4% formaldehyde* solution.

A trend was observed at all dosages toward increased absolute (A) and relative (R) weight of spleen, liver, kidney and pituitary in males, and toward decreased weight of these organs in females.

The increases were 12% (A) and 8% (R) in liver, 21% (A) and 17% (R) in spleen and 23% (A) and 19% (R) in kidneys in males at the 5 ppm level. These increases were significant ($p < .05$) for absolute weights spleen and liver. In females the decrease was 18% (A) and 15% (R) in kidneys at the 5 ppm dosage, $p < .05$ for the absolute weight decrease; the decreases in the liver and spleen weights (A) and (R) were statistically insignificant. It is worthwhile to note, however, the change in these two organs because a clear difference is evident in Di-Syston effects between males and females that seem to be opposite the effect seen in cholinesterase inhibition.

The brain, at all levels tested, showed a trend toward decreased absolute and relative weight in males and toward increased weights in females.

Generally histopathological investigations did not show any specific pathological changes that could be associated with these difference in organ weights in the 5 ppm treated group. The liver and spleen did not show any serious pathological changes in treated animals as compared to the control group.

In summary, pathological changes were observed in the control as well as the treated animals; these changes were considered of spontaneous senile origin common to the laboratory rats.

Tumor Assessment

During this experiment, the following numbers of tumor-bearing animals were sacrificed moribund or found dead. It is clear if these tumors were palpated or noted only at necropsy.

| Dosage (ppm) | Males | Females |
|---|---|---|
| 0.0 | 8 (4) | 22 (4) |
| 0.5/5.0 | 10 (3) | 19 (5) |
| 1.0 | 7 (2) | 17 (8) |
| 2.0 | 12 (1) | 12 (4) |

The figures in brackets specify the number of animals which could not be investigated histopathologically as a result of the progressed autolytic state.

At termination all animals were examined for tumors; it is not clear if these tumors were initially detected macroscopically or microscopically.

The total number of tumor-bearing animals in this study was as follows:

| Dosage (ppm) | Males | Females |
|---|---|---|
| 0.0 | 11 | 25 |
| 0.5/5.0 | 14 | 24 |
| 1.0 | 11 | 21 |
| 2.0 | 16 | 15 |

The number of tumor-bearing animals at all dose levels seems to be comparable to the control group, this may suggest that Di-Syston is not oncogenic. However, due to inadequate data tabulation it is not clear if all animals in this study were grossly examined, or if a relatively large number of animals were autolized.

Dr. L. Kasza (Toxicology Branch Pathologist) concurs with the conclusion that the data as presented did not demonstrate an oncogenic effect for Di-Syston. Dr. Kasza concluded that "in spite of the fact that there is a statement in the report that all animals were inspected for tumors, there is no indication that all animals were checked grossly for neoplasms at all dose levels or that sections were made when neoplasms were found. Based on our review, no data could be found on approximately 50% of the animals in this 2-year chronic feeding/oncogenic study (See table above)".

Dr. Kasza concluded that "the available data are not sufficient to determine whether Di-Syston is oncogenic or not. Therefore it is necessary that another oncogenic study, properly designed and performed be submitted".

Discussions and Conclusions:

Invalid high dose. The increase in dosage from 0.5 to 5 ppm occurred on the 81st week of this study. The dosage of 5 ppm cannot be considered an effective high dose because the change after 81 weeks is not acceptable.

Insufficient Necropsy Data. At termination, only 10 animals/sex/group were necropsied, the remaining animals that died during the experiment (almost 50%) were not reported.

Insufficient Histology Data. At termination, only 5 animals/sex were examined from the control group and the 0.5/5.0 ppm dosage group. No histology was done on animals treated with 1 and 2 ppm dosages because the investigators considered that substance-related histopathological findings were not observed at the high dose of 5.0 ppm. We find this justification inadequate because:

1. The high dose of 5.0 ppm was not a valid high dose because the animals were exposed to this dosage are actually exposed to a time weighted dosage of 1.5 ppm).

2. Histopathology should have been done at least on all animals of the high dose.

No raw data were available for our review, Mobay did not forward these data on request, consequently we could not analyze certain effects. For example, we noticed an average of 75% male mortality at 2 ppm dose level, however we could not assess the cause of death nor its implication because necropsy data for these animals were not available.

Finally, criteria for using specific tests in the statistical analyses should be explained, (B. Litt, Toxicology Branch, Statistician).

In conclusion, the data as presented in this study were inadequate for an oncogenic evaluation.

Considering the inadequacy of the histopathology and necropsy data, a NOEL for systemic toxicity could not be determined.

Based on cholinesterase inhibition in females at 1 ppm (LDT) the NOEL could not be established because at 1 ppm dietary level, brain cholinesterase was inhibited 11% ($p < .01$); and because data on RBCChE inhibition was hindered due to the removal of food 24 hours before termination. The effect of these 24 hours fasting on the reversibility of Di-Syston - ChE inhibition may have reflected a lower ChE inhibition levels.

Core Classification: Supplementary, reports of necropsy on animals that died during the experiments (almost 50%) are needed, report of gross lesions for all the animals at termination are needed as well as histopathology of all gross lesions.



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* This study was originally reviewed by A. Mahfouz, and further evaluated by G. Ghali for re-registration purposes.

003958

Chronic Feeding Study in the Dog

Fiche/Master ID 00073348

Hoffmann, K.; Weischer, C.H.; Luckhaus, G.; et al. (1975) S 276 (Disulfoton) Chronic Toxicity Study on Dogs (Two-year Feeding Experiment): Report No. 5618; Report No. 45287. (Unpublished study received Dec. 15, 1976 under 3125-58; prepared by Bayer, AG, W. Germany, submitted by Mobay Chemical Corp., Kansas City, Mo.; CDL:095640-N)

Test Chemical:

Technical Di-Syston 95.7%.

Experimental Protocol:

Four groups of four male and four female pure bred Beagle dogs, 20-22 weeks old, were maintained on diets containing 0.0, 0.5, 1.0 or 2.0 ppm of the test compound. The first three groups were maintained on these diets for 104 weeks. the high dose group was given 2.0 ppm for the first 69 weeks, then 5.0 ppm from week 70 to 72, and finally 8.0 ppm from week 73 until termination. Food consumption, general appearance and behavior, were recorded daily. The animals were weighed weekly for the first 52 weeks and bi-weekly thereafter. Body temperature, pupillary reflex, patellar reflex, flexor reflex, extensor thrust, ophthalmology examinations, hematology testing (including: hematocrit value, hemaglobin count, erythrocyte count, leucocyte count, medium cell hemoglobin, sedimentation rate, thrombocyte count, and differential blood count), clinical chemistry testing (including: blood sugar, cholesterol, plasma urea, total protein, glutamate-oxalate transaminase, glutamate-pyruvate transaminase, alkaline phosphatase, Bromosulphalin excretion, and phenol red test), and urinalysis (including: Albumin, blood, sugar, pH, specific gravity and microscopical examination of sediments) were performed on all animals before the commencement of the feeding experiment, on weeks 13, 26, 39, 52, 65, 78, 91, and at termination.

Cholinesterase activity including plasma cholinestease and erythrocyte cholinesterase, brain cholinesterase were measured at 2-week intervals during the first 13 weeks and at about 3 month intervals thereafter. Brain cholinesterase was tested immediately after necropsy.

At the end of the experiment (104 weeks), all dogs were narcotized with Evipan and sacrificed by exsanguination. The organs weighed and examined for gross pathology were as follows:

| | | |
|---|---|---|
| Brain | Adrenals | Heart |
| Liver | Pituitary | Lung |
| Spleen | Thyroid | Testes/Ovaries |
| Kidney | Pancreas | Prostate Gland |

The tissues examined for histopathology were as follows:

| | | |
|---|---|---|
| Brain | Adrenals | Testes |
| Liver | Pituitary | Epididymes |
| Spleen | Thyroid | Prostate Gland |
| Kidney | Thymus | Ovaries |
| Heart | Glandula Parotis | Uterus |
| Lung | Mesenteric Lymph Nodes | Gall Bladder |
| Esophagus | Eyes | Urinary Bladder |
| Stomach | N. Optici | Bones (Os femuris) |
| Duodenum | N. Ischiadicus | Bone Marrow (sternum) |
| Jejunum | Aorta | |
| Ileum Colon | Skeletal Muscles (Musculus quadriceps) | |

Bones were decalcified with EDTA, and tissues were routinely processed for histopathological examination.

Results:

No treatment-related effects were observed with respect to general appearance and behavior, and toxic signs. Ophthalmoscopic examinations, food consumption, body weight, hematology, and clinical chemistry testings showed no significant deviation from normal.

Di-Syston did not affect the cholinesterase activity at 0.5 or 1.0 ppm in dogs, the inhibition of PChE, RBC ChE and BChE was insignificant at these dosages after 104 weeks of dietary exposure.

Exposure to 2 ppm dosage for the first 69 weeks of this study caused PChE and RBC ChE inhibition in both males and females. The maximum inhibition was noted at week 40 where males demonstrated a 50% and 33% inhibition of RBC ChE and PChE respectively; and for females, a 22% and 36% inhibition of RBC ChE and PChE was noted. Large fluctuations in the degree of inhibition were noted in both PChE and RBC ChE, at this dosage during the 69 weeks of exposure.

When 2 ppm dosage ws increased to 8 ppm, ChE inhibition data were more consistent during the remaining period of the study where males demonstrated a 56-66% and 63-70% inhibition of RBC ChE and PChE respectively; and for females, a 46-53% and 54-64% inhibition was 34% in males and 18% in females.

In conclusion, these data demonstrate that the NOEL for PChE and RBC ChE is 1 ppm and the LEL is 2 ppm.

Di-Syston treatment did not result in treatment-related organ weight or histopathological changes.

Conclusion:

No compound-related mortality was reported. Behavior growth and all other toxicity parameters were not significantly affected by Di-syston during this experiment.

Based on these data the NOEL for systemic toxicity is 2 ppm. This was the highest dose level for the first 69 weeks of the study.

Dietary concentrations of 1.0 ppm Di-Syston and below did not cause any depression of PChE, RBC ChE or BChE activity compared to the respective control values.

Based on these data, the NOEL for RBC ChE and PChE is 1 ppm and LEL is 2 ppm.

Core Classification:

Core Minimum data.

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This study was originally reviewed by A. Mahfouz, and further evaluated by G. Ghali.

003958

Oncogenicity Study in Mice

Fiche Master ID 000000000

Hayes, R.H. (1983). Oncogenicity study of disulfoton technical in mice. An unpublished report of study No. 80-271-04 prepared by the Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, KS. Dated August 10, 1983.

Test Chemical:

Disulfoton technical 98.2%.

Experimental Protocol:

1. Male and female $CD_1$, outbred strain albino mice were obtained from Charles River Breeding Laboratories. The animals were acclimated to laboratory conditions for a period of one week, then randomly assigned to 4 groups of 50 males and 50 females each. In addition, 10 mice/sex/group were used as replacement animals. Animals were housed individually in stainless steel suspended cages in rooms maintained at 20-23°C, 35-55 percent relative humidity with a 12 hour light/dark cycle. The mice were 6 weeks of age when dosing began.

2. The diets were prepared weekly by mixing the test material in corn oil (one percent by weight) with Ralston Purina Rodent Chow 5001-4 (Etts form) at concentrations of 0, 1, 4, and 16 ppm. The diets were kept in a freezer and portions made available to the animals daily. Food and water available ad libitum during the study.

3. Samples of the prepared diets were collected weekly and stored frozen; one sample per month was analyzed to determine the concentration of the test material. The homogeneity of the test material in the prepared diets was determined by gas-liquid chromatography from three 25-g random samples collected from the top, middle, and bottom of the mixing bowl of the 1 and 16 ppm batches. The stability of disulfoton in the feed was also determined using [$^{14}$C]-disulfoton mixed at a nominal concentration of 4 ppm. Samples were collected following diet preparation and then from the frozen diet on days 7, 14, and 21. The analytically determined concentrations were compared with samples collected on days 10, 14, 17, and 21 from a batch of diet frozen for a week and then kept at room temperature.

4. The animals were observed twice daily for signs of toxicity, moribundity, and mortality; on holidays, observations were made only once. Once each week the animals were palpated for tissue masses. Body weights and food consumption were determined weekly during the study.

5. Hematology data were collected at study months 6, 12, and 23 (termination) from 10 mice/sex/group; different mice were used for each date. Determinations made with the Coulter Counter (model S-plus) include hematocrit, hemoglobin, erythrocyte count, leukocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelets; differential leukocyte counts were made from blood smears stained with Wright's stain.

   Cholinesterase activity in the plasma, erythrocyte, and brain was determined at final sacrifice for 10 mice/sex randomly selected from the control and 16 ppm groups.

6. Necropsy was performed on each animal found dead, sacrificed moribund or sacrificed by carbon dioxide asphyxiation at study termination (23 months). The gross examination included the external surface, all orifices, cranial cavity, "carcass", external cut surfaces of the brain and spinal cord, the viscera of the thoracic, abdominal, and pelvic cavities, as well as the cervical tissues and organs.

7. The weights of the following organs were determined: adrenals, brain and the entire brainstem, gonads, heart, kidneys, liver, lungs, and spleen. Samples of all the tissues and organs required by Subdivision F of the EPA Pesticide Assessment Guidelines of 1982 were collected from all animals and fixed in 10 percent buffered formalin. The fixed tissues were processed and evaluated for histopathological changes by Westpath Laboratories of Fort Collins, CO.

8. Statistical analysis of the data for body weights, feed consumption, hematology, and absolute and relative organ weights was by an analysis of variance followed by a test for the least significant difference or Duncan's New Multiple Range Test. The chi-square statistic and Fischer's Exact Test were applied to the incidences of lesions or tumors. The computer-based statistical analysis used programs from SAS Institute, Inc. of Cary, N.C. The fiducial limits of significance were set at $p \leq 0.05$.

RESULTS:

Analysis of Test Material and Diets: The purity of technical disulfoton at months 1, 6, 12, 18, and 23 of the study was to be 97.8, 98.2, 98.0, 98.1, and 97.8 percent. The results of the stability study with [$^{14}$C] disulfoton showed that the concentrations of the test material in the diet declined 10 percent over the 7 day period the diet was offered to the mice. The test material was found to be evenly distributed in the diets.

The analytical concentrations of disulfoton in the diets throughout the study were as follows:

| Nominal Concentration (ppm) | Analytical Concentrations mean ± S.D. (ppm) | Range (ppm) |
|---|---|---|
| 1 | 0.77 ± 0.13 | 0.05 - 0.99 |
| 4 | 3.55 ± 0.48 | 2.60 - 4.30 |
| 16 | 14.76 ± 1.76 | 11.01 - 17.00 |

Deviations in excess of 25 percent of the dietary target concentration occurred 8 times for 1 ppm (months 0, 1, 2, 4, 6, 8, 15, and 17), twice for 4 ppm (months 1 and 8), and 3 times for 16 ppm (months 0, 1, and 20). However, all supporting data for test material and diet analyses were not presented in the report.

Clinical Observations and Mortality: Limited information on clinical observations was presented. It was stated in the report that "daily observations for toxicological effects (moribundity and mortality) revealed as follows: rough coat, urine stain, balding, eye lesions, exophthalmia, corneal opacity, lesions, nodules, mass, swollen lymph nodes, enlarged abdomen, prolapsed penis, prolapsed rectum, prolapsed vagina, bloody vaginal discharge, and convulsions." Treated animals "did not exhibit a notable increased incidence or dose-related trends for these observations when compared to control groups." However, data on clinical signs of toxicity for individuals or groups of animals were not presented.

Eight mice that died or were moribund during the first month of the study were replaced. These included 1 male and 3 females from the 1 ppm group, 3 males from the 4 ppm group, and one male from the 16 ppm group. There were no significant differences in survival among treated animals as compared to controls throughout the 23-month study. Survival at 18 months ranged from 76 to 86 percent in all male groups and 68 to 82 percent in all female groups. At the end of the study, survival in males ranged from 56 to 66 percent and in females ranged from 38 to 54 percent. Mortality among groups was not dose-related. The average survival time in males ranged from 611 to 641 days and in females ranged from 589 to 618 days.

Body Weights: There were no compound-related differences in mean body weights among treated and control male and female animals. However, the high dose female animals exhibited consistently (42 out of 99 weeks) higher mean body weights than controls after week 8.

Food Consumption: Mean food consumption data indicated some variability among groups but no compound-related effects or trends. There were both significant increases and decreases at different intervals among treated groups when compared to controls.

Hematology: There were some isolated increased or decreased values noted in some parameters among treated and control groups at the 6 and 12 month sampling interval, but not at final sacrifice. However, the changes were not time- or dose-related and were not considered to be related to treamtent.

Cholinesterase Activity: Cholinesterase activity in plasma, erythrocytes, and brain were determined only in control and high-dose animals at final sacrifice. A significant decrease in activity was noted in both males and females as compared to controls (Table 1).

TABLE 1. Mean Cholinesterase Activity in Mice Fed Disulfoton Diets for 23 Months

| Test Group | Mean Cholinesterase Activity (umole/ml/min) Plasma | Erythrocyte | Brain |
|---|---|---|---|
| Males | | | |
| Control | 4.07 | 0.91 | 14.45 |
| 16 ppm | 0.84 (79[a]) | 0.40 (56) | 7.97 (44) |
| Female | | | |
| Control | 4.85 | 0.85 | 14.04 |
| 16 ppm | 0.86 (82) | 0.43 (50) | 7.62 (46) |

[a]percent inhibition.

Gross Pathology: Gross findings for individual animals and tissues were presented together with the correlative histopathologic diagnoses for all animals. Summary group data were not presented. The data indicated that the most frequently gross observation in male and female mice was organ enlargement, particularly the spleen, liver, and lymph nodes at various locations. The corresponding histopathologic diagnosis was malignant lymphoma of these organs. The incidence was greater in females (see also Histopathology). Enlarged uteri containing cysts were often noted which were diagnosed as cystic glandular hyperplasia of the uterine endometrium.

Similarly, a large number of ovarian follicular cysts were noted. A number of animals had masses uterine horns and cervix which were diagnosed as being uterine leiomyoma. Reduction in size or loss of the eye associated with phthisis bulbi was also noted in a small number of mice. The incidences of the above-mentioned lesions were similar among treated and contorl animals, and none were considered to be related to the test material (see also Histopathology).

Organ Weights: A significant increase in mean kidney weight and kidney to body weight ratio was noted in the high dose female group when compared to control. In addition, there was a dose-related decrease in the mean treated groups (Table 2). Individual data indicated that large ovarian cysts were

present in five control mice causing an increase in the mean ovarian weight of the control group. Correcting the weights for the cystic ovaries decreased the mean from 2.735 to 0.811 g. The authors also stated that "analysis of the number of weights of non-cystic ovaries (twelve control, fifteen 1 ppm, eleven 4-ppm, and sixteen 16-ppm) showed no significant differences in weights or number of mice with non-cystic ovaries between compound test groups and controls." However, the animals were not identified. A validation of this statement by this reviewer identified ten control and fifteen 16-ppm females without ovarian cysts; the mean ovary weights were determined to be 0.031 and 0.05 g, respectively.

An increase in the lung, liver, and adrenal weights relative to body weights were also noted in the mid-dose females. There were no changes in organ weights and organ to body ratios in treated males.

TABLE 2. Mean Organ Weights and Organ to Body Weight Ratios in Female Mice Fed Disulfoton

| Test Group | Kidney | | Ovary | |
|---|---|---|---|---|
| | (g) | (percent) | (g) | (percent) |
| Control | 0.582 | 1.720 | 2.735 | 5.911 |
| 1 ppm | 0.534 | 1.805 | 0.714* | 1.827* |
| 4 ppm | 0.597 | 1.812 | 0.693* | 1.928* |
| 16 ppm | 0.651* | 1.917* | 0.625* | 1.604* |

*$p < 0.05$.

Histopathology: Individual and group histopathologic findings were presented for all animals. The neoplasms observed most frequently in this study as summarized in Table 3. The incidence of specific neoplasms were similar among dosed and control animals. The number of animals with malignant lymphoma, of all histologic cell types, was found to be 10, 9, 12, 15, in male mice and 27, 22, 26, 34 in female mice at

TABLE 3. Summary of Neoplastic Lesions Observed Most Frequently in Mice

| Neoplastic Lesions | Dose Group (ppm) Sex: | Control M | Control F | 1 M | 1 F | 4 M | 4 F | 16 M | 16 F |
|---|---|---|---|---|---|---|---|---|---|
| Aorta | N[a] | 48 | 46 | 50 | 50 | 50 | 49 | 50 | 50 |
| Lymphoma | | 0 | 6 | 5 | 4 | 1 | 5 | 6 | 7 |
| Liver | N | 49 | 49 | 50 | 49 | 50 | 49 | 50 | 50 |
| Adenoma | | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Carinoma | | 8 | 1 | 7 | 1 | 11 | 0 | 8 | 0 |
| Lymphoma | | 5 | 9 | 6 | 9 | 3 | 11 | 9 | 10 |
| Adrenal glands | N | 47 | 50 | 50 | 49 | 46 | 47 | 49 | 50 |
| Lymphoma | | 1 | 2 | 4 | 2 | 1 | 2 | 5 | 4 |
| Bone Marrow | N | 47 | 50 | 48 | 43 | 48 | 45 | 49 | 49 |
| Lymphoma | | 3 | 11 | 5 | 5 | 3 | 5 | 9 | 6 |
| Spleen | N | 47 | 50 | 49 | 49 | 48 | 48 | 48 | 47 |
| Lymphoma | | 7 | 18 | 7 | 19 | 9 | 17 | 12 | 23 |
| Thymus | N | 30 | 43 | 32 | 43 | 39 | 40 | 30 | 47 |
| Lymphoma | | 4 | 13 | 6 | 10 | 4 | 11 | 8 | 26 |
| Lung | N | 49 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Adenomas | | 2 | 4 | 3 | 2 | 2 | 1 | 3 | 4 |
| Carinoma | | 10 | 6 | 9 | 5 | 10 | 4 | 8 | 5 |
| Lymphoma | | 2 | 8 | 0 | 9 | 2 | 10 | 6 | 12 |
| Kidney | N | 49 | 50 | 49 | 49 | 49 | 50 | 48 | 50 |
| Lymphoma | | 3 | 7 | 4 | 8 | 0 | 8 | 2 | 19 |
| Mammary Gland | N | - | 50 | - | 50 | - | 47 | - | 49 |
| Adenocarcinoma | | | 0 | | 1 | | 6 | | 2 |
| Lymphoma | | | 4 | | 2 | | 3 | | 4 |
| Cervix uteri | N | - | 46 | - | 47 | - | 46 | - | 45 |
| Leiomyoma | | | 3 | | 2 | | 4 | | 4 |
| Lymphoma | | | 2 | | 1 | | 2 | | 1 |
| Corpus uteri | N | - | 50 | - | 48 | - | 46 | - | 48 |
| Leiomyoma | | | 11 | | 11 | | 5 | | 6 |
| Lymphoma | | | 2 | | 1 | | 2 | | 2 |
| Ovariesuteri | N | - | 48 | - | 49 | - | 49 | - | 50 |
| Cystadenoma | | | 3 | | 4 | | 2 | | 2 |
| Lymphoma | | | 3 | | 3 | | 4 | | 4 |
| Granulosa cell tumor | | | 2 | | 1 | | 1 | | 3 |

[a]Number of animals with indicated tissue examined microscopically.

dietary levels of 0, 1, 4, and 16 ppm, respectively. Although there was an increased incidence of malignant lymphomas in both males and females at 16 ppm when compared to controls, this was not statistically significant by either the Chi-square or Fisher extact test. An increased incidence of mammary gland carcinoma was also noted in the mid-dose females. Non-neoplastic lesions observed most frequently are summarized in Table 4. The incidences of these lesions in dosed animals were not significantly increased as compared to those observed in the controls. However, a small increase in the incidence of atrophy of the testes coupled with a reduced sperm count in the mid- and high-dose males was noted.

DISCUSSION:

The results of plasma, erythrocyte, and brain cholinesterase inhibition suggest that the highest dose used of the test material approached a maximum test dose level, since significant inhibition of activity was noted. Although, cholinesterase determinations were made on the high-dose and control animals only and the limited data on clinical signs of toxicity presented did not indicate cholinergic effects, the dosage received by the animals by the animals on the 16 ppm diets during the first week, approximate 35 percent of the $LD_{50}$ value (as determined from other studies). These dosages were calculated from food consumption and body weight data and were found to be 3.2 and 3.4 mg/kg for males and females, respectively. Consequently, it is considered that higher dietary concentrations of the test material would have resulted in significant compound-related mortality of the test animals.

There were no significant compound-related effects noted in body weight gain, food consumption, and hematology. Organ weight data, on the other hand, indicated lower weights for the ovaries in all treated groups when compared to controls. However, the mean weights of ovaries in control animals were excessively high because of large ovarian cysts present in 5 mice. Moreover, when the mean ovary weights were determined for those animals without cysts, there was no significant differences between treated and control animals. A significant increase in the mean kidney weight was also noted in the high-dose female group. This increase may be associated with an apparent increased incidence of malignant lymphomas of the kidneys in this groups (7/50, 8/49, and 8/50, respectively). However, this increase in female kidney lymphomas was not statistically significant.

TABLE 4. Summary of Non-Neoplastic Lesions Observed Most Frequently in Mice

| Neoplastic Lesions | Dose Group (ppm) Sex: | Control M | Control F | 1 M | 1 F | 4 M | 4 F | 16 M | 16 F |
|---|---|---|---|---|---|---|---|---|---|
| Liver | N[a] | 49 | 49 | 50 | 49 | 50 | 49 | 50 | 50 |
| necrosis | | 7 | 4 | 0 | 6 | 4 | 1 | 6 | 4 |
| inflammation, chronic | | 43 | 10 | 1 | 8 | 6 | 6 | 3 | 15 |
| Stomach | N | 48 | 49 | 48 | 49 | 50 | 48 | 50 | 50 |
| inflammation, chronic | | 15 | 7 | 11 | 6 | 9 | 13 | 12 | 13 |
| polyp, adenomatous | | 17 | 13 | 13 | 17 | 17 | 25 | 17 | 23 |
| Adrenal gland | N | 47 | 50 | 50 | 49 | 46 | 47 | 49 | 50 |
| congestion | | 0 | 9 | 0 | 13 | 0 | 11 | 0 | 8 |
| deposition, lipofuscin | | 0 | 12 | 0 | 12 | 0 | 19 | 0 | 15 |
| hyperplasia, subcapsulcar | | 15 | 49 | 39 | 46 | 34 | 46 | 22 | 47 |
| Thyroid | N | 46 | 48 | 46 | 48 | 46 | 48 | 44 | 48 |
| cyst, follicular | | - | 8 | 6 | 7 | 9 | 3 | 3 | 4 |
| Lymph node, Mesenteric | N | 46 | 46 | 38 | 39 | 39 | 44 | 45 | 48 |
| hyperplasia | | 2 | 10 | 3 | 8 | 11 | 10 | 2 | 13 |
| telangiectasis | | 5 | 7 | 5 | 9 | 9 | 15 | 8 | 14 |
| Spleen | N | 47 | 50 | 49 | 49 | 48 | 48 | 48 | 47 |
| hyperplasia | | 7 | 5 | 0 | 6 | 4 | 2 | 6 | 4 |
| Epididymides | N | 47 | - | 49 | - | 48 | - | 49 | - |
| reduced sperm count | | | - | 10 | | 14 | | 13 | |
| Testes | N | 48 | - | 49 | - | 48 | - | 50 | - |
| atrophy | | 6 | | 9 | | 13 | | 13 | |
| tabular degeneration | | 14 | | 10 | | 18 | | 18 | |
| Corpus uteri | N | - | 50 | - | 48 | - | 46 | - | 48 |
| hyperplasia | | | 49 | | 42 | | 42 | | 47 |
| Ovaries | N | - | 48 | - | 49 | - | 49 | - | 50 |
| follicular cyst | | | 38 | | 30 | | 37 | | 36 |
| hematocyst | | | 16 | | 10 | | 12 | | 8 |
| Lung and Bronchi | N | 49 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| atelectasis | | 0 | 9 | 11 | 13 | 6 | 4 | 1 | 7 |
| inflammation, chronic | | 4 | 0 | 12 | 1 | 11 | 6 | 13 | 4 |
| Eye | N | 48 | 49 | 50 | 46 | 50 | 47 | 48 | 47 |
| inflammation, acute | | 1 | 2 | 6 | 0 | 3 | 0 | 4 | 0 |
| inflammation, chronic | | 1 | 1 | 7 | 0 | 1 | 0 | 5 | 0 |
| phthisis bulbi | | 2 | 1 | 3 | 3 | 2 | 1 | 4 | 0 |
| Harderian gland | N | 48 | 50 | 50 | 50 | 49 | 50 | 50 | 50 |
| inflammation, chronic | | 9 | 13 | 19 | 12 | 10 | 11 | 7 | 17 |
| Kidney | N | 49 | 50 | 50 | 49 | 50 | 50 | 50 | 50 |
| cortical cyst | | 13 | 1 | 7 | 5 | 12 | 2 | 10 | 3 |
| cortical fibrosis | | 11 | 2 | 4 | 2 | 11 | 2 | 5 | 3 |
| inflmmation, acute | | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |
| inflammation, chronic | | 35 | 35 | 37 | 21 | 49 | 32 | 29 | 25 |

[a]Number of animals with indicated tissue examined microscopically.

Although survival in some groups at the end of the 23-month study was low, there were no significant differences in survival among treated animals as compared to controls throughout the study. Survival in all groups at 18 months ranged between 68 and 86 percent, thus permitting an assessment of possible late-developing lesions. The most frequently observed neoplastic lesion noted was malignant lymphoma, with the incidence being higher in the females. However, the incidences were similar among treated and control animals. There was a slight increase in the incidence of atrophy of the testes which was also coupled with a reduced sperm count in the mid- and high-dose males; however, the increased incidence of these lesions was not statistically significant.

CONCLUSIONS:

Under the conditions of this 23-month feeding study, disulfoton was not oncogenic to $CD_1$ male and female mice at dietary concentrations of up to 16 ppm.

CORE CLASSIFICATION: Minimum.

Three Generation Reproduction Study on the Rat

Fiche/Master ID 00091104

Taylor, R.E. (1966) Letter sent to D. MacDougall dated May 5, 1966: Di-Syston, three generation rat breeding studies: Submitter 18154. (Unpublished study received Mar. 7,1977 under 3125-252; prepared by Harris Laboratories, Inc., submitted by Mobay Chemical Corp., Kansas City, Mc.; CDL:096021-L)

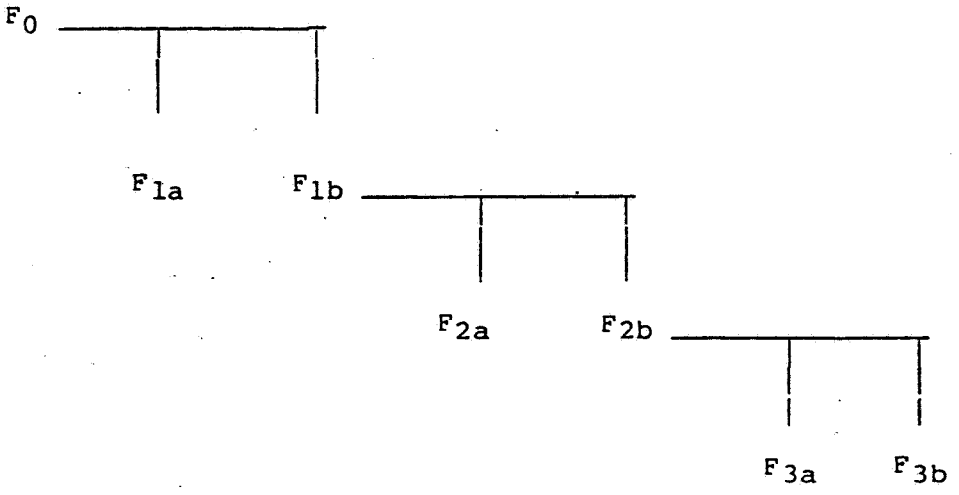
Test Chemcial:

Technical Di-Syston 98.5%.

Experimental Protocol:

Weanling albino Holtmann strain rats (21 days old) were acclimated for 9 days, prior to grouping into control, 2, 5, gand 10 ppm groups. When the animals were 100 days old in each generation, 20 females were mated to 10 males to produce $F_a$ litters. Females were individually housed prior to parturition until the litter was 21 days old.

Ten days following the weaning of $F_a$ litter, parent rats were again mated to produce $F_b$ liter. $F_b$ litters were used for the selection of parents for the next generation according to the following diagram.



Litters were usually culled to 10 on the fifth day after delivery in each mating (see breeding diagram).

Technical Di-Syston was mixed into the diet at a concentration of 0.05% and stored at 38°F. The diets were prepared each 10-12 from the 0.05% premix by adding standard rat feed.

The $F_a$ generations were always weighed, observed, weaned and then discarded.

Feed consumption was determined by weighing feed consumed by each group during a three day period once each month. Usually weight and feed consumption measurements were initiated after the rats were treated for one month. These data were not recorded during the third month of testing as the rats were mating.

Observations were recorded for the number of pregnancies, litter size, survival to weaning day, and pup weights at 21 days.

Necropsy and Histopathology: The investigators reported that after the selection of parents for the second generation from $F_{1b}$ litters, all other rats in $F_{1b}$ litters and parent stock ($F_0$) were necropsied; necropsy was also done on half of the parent stock in the second generation ($F_1$) and the third generation ($F_2$), and on all 21 day old $F_{3b}$ litters. Tissues were removed from necropsied animals in the seond and third generation for possible histopathological examination.

Liver, kidney, adrenal, thymus, and testes or ovaries were histopathologically examined, but not weighed.

Erythrocyte cholinesterase (RBC ChE) activity was measured for all animal groups, male and female separately, of $F_{3b}$ litters and their parent $F_{2b}$ parent. Dubois et al. (1967) manometric technique was used in these determinations.

Statistical Method: None reported.

Results:

Food consumption and body weight gain: body weight was reported for only weighed at age 21 days.

In the first generation, females exposed to 2 ppm, 5 ppm, and 10 ppm dietary levels consumed 25-40% less food than the control group during the first month: during the second month, only the females exposed to 10 ppm dietary level consumed 28% less food than the control group. No weight was observed in association with this reduced food consumption.

Generally, the food intake and weight of animals did not seem to be affected by Di-Syston in the next two generations. A trend toward increased weight gain in adult females of 2nd and 3rd generations was noted at 5 and 10 ppm; as compared to controls, this increase was about 25% at both dosages in each of the two generations.

A similar trend toward increase in weight was noted in $F_{3a}$ and $F_{3b}$ litters at all dosages in the third generation.

Reproduction Data: No major differences were observed in the reproduction parameters at dietary levels of 2 and 5 ppm.

No major differences were observed in the weight of pups at these dietary levels in the first or second generation. However in the third generation the weight of pups at weaning increased as compared to the control groups: at 2 ppm dietary level - the increase was 13% in $F_{3a}$ 9% in $F_{3b}$; at the 5 ppm dietary level - the increase was 2[illegible] in $F_{3a}$; 37% in $F_{3b}$. The significance of the findings is not understood.

At 10 ppm dietary level, in the first generation, the most significant reproductive effects were noted in the $F_{1a}$ litter at this 10 ppm dietary level.

The pregnancy rate was lower than the control group by 25% in $F_{1a}$; the litter size lower 22%; the viability index lower by 83%; and the pup weights at weaning were 16% higher.

The only effect observed in $F_{1b}$ was 30% reduction in litter size as compared to the control group.

However the data on at this dietary level may be hindered by 40% lower food consumption than the control group; the large decrease 83% in $F_{1a}$ viability index may be more by due to food avoidance by the dams than any other factor.

In this generation the dams cannibalized their young at age 15 days; the lactation index could not be determined for this generation because the report did not indicate if the dams cannibalized $F_{1a}$ or $F_{1b}$ litters.

In the second generation, no difference was noted in any reproduction parameter or weight of pups at weaning in either $F_{2a}$ or $F_{2b}$ litters at the 10 ppm dietary level.

In the third generation, as was noted in the first generation, the litter size in this third generation was lower by 20% in $F_{3a}$ and 36% in $F_{3b}$ at 10 ppm dietary level as compared to the control groups. Similarly a 10% lower rate was noted in the first mating.

The assessment of Di-Syston effect on reproduction from these data would be hindered because of the absence of the following:

- The number of pups alive and stillborn per litter
- The weight of pups at birth and the weekly rate of their growth until weaning
- The statistical analysis of submitted data

In spite of the above listed inadequacies, the 10 ppm dietary level reflected the average reduction in litter size of 21% in $F_{1a}$ and 33% in $F_{1b}$ in both the first and 3rd generation.

Blood Cholinesterase: data on RBC Cholinesterase activity were reported on the third generation for $F_{2b}$ adults and their $F_{3b}$ litters; the number of tested animals was not indicated.

RBC ChE inhibition was significant in both parents and litters; this inhibition was within the same range for parents and litters of the same sex at a given Di-Syston dietary level; the data for this inhibition were as follows:

2 ppm dietary level - 32-42% (F); 10-13% (M)
5 ppm dietary level - 67-68% (F); 52-46% (M)
10 ppm dietary level - 70-78% (F); 69-70% (M)

Based on these data the NOEL for RBC ChE inhibition in males is 2 ppm; in females the LEL = 2 ppm.

Gross Pathology and Mortality: it is clear from the above that the necropsy was done on $F_{3b}$ litters, their parents, and the animals that died during the study.

In the first generation, on the control, 2, 5, and 10 ppm dietary levels, 6, 5, 3, and 5 dams respectively killed their young at 15 days age, and half of the remaining litters died five days after weaning reportedly due to an overnight failure of the air conditioning system. No gross pathology was reported on this generation.

In the second generation, at 2 ppm dietary level, one female had panophthalmia in one eye; a second female was comatose and post mortem examination revealed acute dilation of the stomach due to hemorrage. No other abnormalities were noted. No gross pathology data were reported on the parental generation or their litters at termination.

In the third generation, at 10 ppm dietary level, one female died and gross findings indicated that death was due to hemorrhagic colitis and pyonephritis. At termination of the $F_{3b}$ parents, one female control exhibited edema of the omentum and generalized emaciation; and some abnormalities were observed at the 2 ppm dietary level:

- Atrophy of the testes in one male
- Paleness of the visceral organs and slight emaciation in two females
- Light brown discoloration of the liver and petechial hemorrhage of the small intestine in one female

The data did not reflect any further visceral effects at 5 ppm or 10 ppm dosages which indicates that the effects seen at the 2 ppm dietary level may not be Di-Syston related.

In $F_{3b}$ litters, two females at 5 ppm dosage showed paleness of visceral organs and slight emaciation. One female from the control showed cystic ovary. No significant gross pathological changes were observed in the remaining animals of this generation.

However histopathological examinations of 10(F) and 5(M) of these litters showed effects on testes, liver and kidneys at the 10 ppm dietary level (see histopathology). No histological data were available at 2 ppm or 5 ppm, and hence these findings could not be evaluated at lower levels.

Absolute and relative organ weights were not reported.

Histopathology: in $F_{3b}$ litters, the 10 ppm dosage group showed the following effects:

Juvenile hypoplasia in testes of 5/5 males as compared to 0/5 in control animals.

Cloudy swelling of the liver with fatty metamorphosis is 4/5 males and 10/10 females as compared to mild liver congestion and vacuolization in control animals 4/5 males and 4/10 females.

Swollen congested cortex, occasional fatty degeneration of the convoluted tubules, or mild chronic nephritis in kidneys of 3/5 males and 6/10 females as compared to mild focal nephritis in kidneys of control animals 1/5 males and 3/10 females.

No data were available at 2 or 5 ppm dosages.

The data as presented reflected a toxic effect in $F_{3b}$ litters on testes, liver and kidneys at 10 ppm. However, due to small number of animals (5M and 10F) histopathologically examined at this 10 ppm dietary level; and due to the absence of any histology data at the lower dietary levels, the systemic toxicity and the effect of Di-Syston on the male reproductive organs could not be fully assessed from this study.

Conclusions:

The reproduction parameters reported in this study were not affected by 2 ppm and 5 ppm dietary levels. At 10 ppm dietary level, the litter size was reduced by 21% in $F_a$ and 33% in $F_b$ in both the 1st and 3rd generation; in these two generations 10-25% lower pregnancy rate was also noted for $F_a$ matings. The viability index for $F_{1a}$ decreased by 83% as compared to the control group in the first generation, however, these data for $F_{1a}$ are hindered by the noted 40% reduction in food intake.

Histopathologically, the 10 ppm dietary level of Di-Syston resulted in 100% juvenile hypoplasia in the five males examined in $F_{3b}$ litters as compared to none in the five males examined in the control group. However, these data are hindered by the small number of animals examined in the litters and adult groups.

Based on these data no NOEL could be determined for Di-Syston effect on reproduction from this study.

60-70% inhibition of RBC ChE was noted in $F_{3b}$ litters and their parents at 5 ppm and 10 ppm dosage, at 2 ppm the inhibition was insignificant in males and moderate 30-40% in females.

Core Classification:

This study is classified as Core-supplementary data.

The data as presented did not properly reflect all reproduction parameters to assess the effect of Di-Syston i.e., the number of pups alive and stillborn per litter was not reported; the weight of pups at birth and their weekly growth rate until weaning was not reported.

Evaluation of data from the first generation was complicated by cannibilism and a failure of the air conditioning system; this failure resulted in the death of many litters.

Complete necropsy was not reported on $F_{3b}$ litters and histopathology was done on 10 (F) and 5 (M) of $F_{3b}$ litters and their parents at only the 10 ppm dose level and the control group. Although compound related effects were noted at 10 ppm level, histopathology was not done on the lower dietary levels.

The statistical analysis of the data was not done.

Considering the deficiencies and inadequacies discussed, a NOEL for reproductive effects could not be determined from this study. Therefore, another 3-generation rat reproduction study must be submitted.

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The study was originally reviewed by A. Mahfouz and further reevaluated by G. Ghali for reregistration purposes.



[redacted]

Teratology Study in Rats

Fiche Master ID 000000000

Lamb, D.W. and Hixson, E.J. (1983). Embryotoxic and teratogenic effects of Disulfoton. An ubpublished report on Study Number 81-611-02 submitted by Mobay Chemical Corp. May 13, 1983.

Test Chemical:

Disulfoton technical 98.2%.

Experimental Protocol:

1. One hundred seventy 50- to 60-day old female CD rats supplied by Charles River Breeding Laboratories, Inc. were used for the study. An unspecified number of adult male CD rats, received from the same supplier and maintained as a breeding colony were used for mating purposes only. The females were acclimated to laboratory conditions for seven day prior to study initiation.

2. Disulfoton was suspended in a Carbowax (polyethylene glycol 400) vehicle to provide dose levels of 100, 300, and 1000 ug/kg/day. The rationale for selecting the dosaghes was not stated. A positive control group received hydroxyurea at 350 mg/kg at 0.25 percent of the body weight on days 9, 10, and 11 of gestation. The dosages of the vehicle and test material were administered daily by gavage at 0.25 percent of the body weight from day 6 to 15 of gestation. Dose volumes were based on the day 6 of gestation body weights.

   The dosing solutions were analyzed for disulfoton content by gas chromatography approximately weekly. The disulfoton content of the analyzed solutions was reported to range from 74 to 106 percent of nominal content. A stability test determined that the stock solution from which the dosing solutions were prepared was stable for 21 days at room temperature.

3. The rats were housed in a room controlled for temperature (69-74°F), relative humidity (35-55 percent), and light (12 hour light/dark cycle). The females were individually housed in stainless steel wire mesh cages. Purina Laboratory Rodent Chow and water were available ad libitum.

4. The females were mated to the males overnight, but the method of selecting mating pairs, the ratio of females to males, and the duration of mating were not stated in the Mobay report. The females were examined each morning for the presence of sperm in a vaginal smear and to determine the stages of estrus. The day that sperm were found was designated at day 0 of gestation, at which time the females were randomly assigned to treatment groups using a computer generated weight-stratified design. Twenty-five mated females were assigned to each of the five treatment groups.

5. Indivdual body weights were recorded on gestation days 0, 6, 13, 16, and 21. Food consumption was determined for gestation days 0-6, 6-13, and 13-21. The dams were observed daily for appearance, health, and evidence of "abortion" and premature delivery. Plasma and RBC cholinesterase activities were determined in five dams/group bled by orbital sinus puncture on day 15 of gestation. An automatic blood chemistry analyzer was used to determine the enzyme activities. The Mobay report did not specify the method employed to select the dams.

6. The rats were sacrificed on day 21 of gestation by carbon dioxide asphyxiation and examined for gross lesions. The uterus and ovaries were removed in toto and weighed. The ovaries were examined for the number of corpora lutea and the uterus was opened and the contents were examined. The number and distribution of live, dead, and resorbed fetuses were determined. The placentas were examined for gross abnormalities and each fetus was weighed, sexed, and examined for gross external abnormalities.

   Fifty percent of the fetuses in each litter were selected by an unspecified method and were fixed in Bouin's solution. After fixation, the fetuses were examined for soft-tissue abnormalities by Wilson's technique. The remaining fetuses were fixed in 70 percent ethanol, examined, internally, eviscerated, prepared for staining with Alizarin Red S, and following destaining of nonossified tissue, were examined for skeletal abnormalities.

7. A Chi-square analysis utilizing SAS computer software was used to test body weights, food consumption, litter data, and external, soft tissue, and skeletal abnormalities for statistical significance. The fetal malformation data were analyzed for each individual abnormality and total abnormalities using both the fetus and the litter as the basic sample unit. Unit otherwise stated, the use of the word "significant" in this evaluation implies a statistical connotation with $p < 0.05$ as reported by Mobay.

RESULTS:

Clinical Observations and Mortalities: Alopecia was observed on two dams in the 100 ug/kg dose group. No abnormal clinical observations were observed among the remaining dams. There were no deaths prior to sacrifice on day 21 of gestation and at necropsy of the dams, no gross lesions were observed except for a pale liver and mottled kidneys in one female receiving 100 ug/kg disulfoton and mottled kidneys in a dam receiving 300 ug/kg disulfoton.

Body Weights and Food Consumption: The days 0, 6, 13, and 21 of gestation mean body weights of disulfoton-treated dams were similar to the negative control dams. Mean corrected body weight gains were similar among the disulfoton and negative control groups; however, this correction was not defined. While it is understood that the maternal body weight was "corrected" by subtracting the gravid uterus weight, the gestation day to which it was corrected was not indicated.

Food consumption was similar among the disulfoton and negative control groups during the three intervals.

Group mean data were not presented for the positive controls.

Cholinesterase Activities: The mean plasma and RBC cholinesterase activities for the dams receiving 300 and 1000 ug/kg disulfoton were significantly depressed when compared to the negative controls. Plasma cholinesterase activity, compared to the negative controls, was depressed 41 and 90 percent for the 300 and 1000 ug/kg groups, respectively. The low dose group RBC (9 percent) and plasma (6 percent) cholinesterase activities were similar to the negative control group.

Reproductive Indices: A summary of the reproduction indices is presented in Table 1. A comparison of the mean numbers of corpora lutea, early resorptions, late resorptions, dead fetuses, and mean fetal body weights indicated no differences between the disulfoton dose groups and the negative controls. The positive control was similar to the negative control for these parameters.

A slight, non-significant decrease in the mean numbers of implantation sites and live fetuses per litter was observed when the disulfoton and positive control dams were compared to the control. The mean numbers of fetuses/litter with gross external abnormalities were similar between the disulfoton and negative control groups while the positive controls had a greater than 10-fold increase when compared to the negative controls.

Fetal Evaluation: The incidences of major malformations and malformations affecting more than one litter are presented in Table 2. Major malformations are defined as malformations that are not comparable with life or that occur rarely in Sprague-Dawley rats.

Hydroxyurea, the positive control, produced increased external (raised cranium and reduced eye bulges), soft tissue (micropthalmia, hydrocephalus, and depressed olfactory bulbs), and skeletal (reduced cranial ossification, lobed and split vertebral centra, and extra ribs) abnormalities when compared to the negative control.

The incidences of external and soft tissues abnormalities were similar when the disulfoton-group fetuses and litters were compared to the controls. In the 300 ug/kg dose group the occurrence of micropthalmia, exencephaly, and hydrocephaly was restricted to one litter and, although not vertifiable, probably one fetus.

A slight, nonstatistically significant increase when compared to the negative contorls was observed in the number of high dose fetuses and litters with extra ribs and with incomplete ossification of the intraparietals.

The numbers of fetuses and litters with lobed or split vertebral centra were increased non-significantly in the three disulfoton dose groups. This increase did not appear to be related to the dosage. A significant increase in the number of fetuses with incompletely ossified sternebrae was observed at the high dose level, but the Mobay report did not indicate whether the number of affected high dose litters (6) also was significantly greater than the negative controls (3 litters).

DISCUSSION:

Oral administration of disulfoton did not produce any overt signs of maternal toxicity. The test material produced significant decreases in RBC and plasma cholinesterase activities of maternal animals. Although the decreases in the enzyme activities were not manifested clinically, the extent of enzyme inhibition strongly supports the consideration that higher doses than 1000 ug/kg could have been lethal to the dams. Consequently, it is considered that a maximum acceptable test dose was used.

There were no statistically significant effects on the reproductive parameters that were examined. However, despite the absence of significance, there was a decrease in the number of implantation sites/litter among the disulfoton-dosed dams. Implantation occurs on day 6 of gestation, the first day of dose administration, which indicates that the pre-implantation losses may be related to disulfoton. However, this sems unlikely for two reasons. A dose-related increase in pre-implantation losses was not observed, and the ratio of implantation sites to corpora lutea determined in the disulfoton-treated groups was not usual for Sprague-Dawley rats. Indeed, the pre-implantation loss observed among the negative controls is small than normally encountered and this gives rise to the appearance of an increased pre-implantation loss among the disulfoton dams.

The nonsignificant decrease in the numbers of live fetuses/litter observed among the disulfoton-dosed dams does not appear to be compound-related. The observed decrease resulted from the previously discussed decreased in implantation sites. A disulfoton-induced embryolethality would be expected to have manifested itself by a decrease in live fetuses/litter and a concurrent increase in resorptions or dead fetuses per litter.

Disulfoton did not produce increased incidences of soft tissue or external abnormalities in the fetuses. Among the 1000 ug/kg fetuses, increased incidences of incompletely ossified parietal bones and sternebrae were observed. A statistically significant increase in the number of 1000 ug/kg litters with fetuse having incompletely ossificated sternebrae was also observed.

These abnormalities, while apparently related to the test material are considered to be indicative of retarded development and, therefore, a fetotoxic effect rather than a teratogenic effect.

A slight, nonsignificant increase in the number of 1000 ug/kg fetuses with extra ribs was observed. In the absence of any major abnormalities among the dose group fetuses, the slight increase in extra ribs did not clearly indicate a disulfoton-related teratogenic effect on skeletal formation.

Inconsistencies were detected in the summary data for fetal mean body weights presented in the Mobay report. The fetal mean body weights given in Table VI (Cumulative Litter Data) of the Mobay report did not always agree with the values given in Table 3A (Litter Data Summary) or with the mean fetal body

weights calculated by this reviewer from the individual fetal weights. For example, 4.9 g was reported in Table VI as trhe fetal mean body weight for the negative controls while the Table 3A value and this reviewer's calculation from the individual data both agreed on a mean of 5.6 g. Furthermore, the mean fetal weights for the 100 and 1000 ug/kg groups, and the positive control group presented in Table VI and Table 3A of the report did not agree with the means calculated from the individual data. The tabulated fetal mean body weight data presented in this evaluation (Table 1) were recalculated and corrected by this reviewer from individual animal data.

CONCLUSIONS:

Under the conditions of this study, the oral administration of 1000 ug/kg of disulfoton daily from days 6 to 15 of gestation did not produce teratogenic effects in the fetus of the Sprague-Dawley rat. Based on the incomplete ossification of the parietals and sternebrae, disulfoton was demonstrated to be fetotoxic with an LEL of 1000 ug/kg and a NOEL at 300 ug/kg.

CORE CLASSIFICATION: Minimum Data.

TABLE 1. Summary of Reproduction Indices

| | Dose Level (µg/kg) | | | | |
|---|---|---|---|---|---|
| | Negative Control | Positive Control[a] | 100 | 300 | 1000 |
| Dams inseminated | 25 | 25 | 25 | 25 | 25 |
| Dams pregnant | 22 | 24 | 24 | 24 | 24 |
| Dams delivering early | 1 | 0 | 0 | 0 | 0 |
| Dams c-sectioned | 21 | 24 | 24 | 24 | 24 |
| Mean no. of corpora lutea | 14.7 | 14.6 | 14.5 | 14.6 | 14.4 |
| Mean no. of implants | 14.3 | 13.4 | 13.3 | 12.8 | 13.4 |
| Mean no. of early resorptions | 0.9 | 0.9 | 0.6 | 0.7 | 0.7 |
| Mean no. of late resorptions | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| Mean live fetuses/litter | 13.4 | 12.3 | 12.6 | 11.8 | 12.7 |
| Mean fetal body weight (g)[b] | 5.6 | 5.1 | 5.3 | 5.6 | 5.5 |
| Mean fetuses/litter with external abnormalities | 0.4 | 5.1 | 0.5 | 0.3 | 0.5 |

[a] 350 mg/kg hydroxyurea on days 9, 10, and 11 of gestation.

[b] Mean weights calculated from the individual fetal body weight by Dynamac.

TABLE 2. Incidences of Major Fetal Abnormalities and Minor Abnormalities Affecting Greater than One Litter

| | Dose Level (µg/kg) | | | | |
|---|---|---|---|---|---|
| | Negative Control | 100 | 300 | 1000 | Positive Control[a] |
| I. External | | | | | |
| Hematoma | 7/281(4)[b] | 7/302(5) | 4/283(4) | 10/305(6) | 8/296(5) |
| Runts | 0/281 | 3/302(3) | 1/283(1) | 1/305(1) | 2/296(2) |
| Total fetuses affected[c] | 9/281 | 12/302 | 7/283 | 11/305 | 123/296 |
| Total litters affected[c] | 6/21 | 10/24 | 6/24 | 7/24 | 23/24 |
| II. Soft Tissue | | | | | |
| Blood in nasal cavity | 0/142 | 8/144(6) | 3/136(2) | 5/146(3) | 4/140(2) |
| Esophagus distended | 3/142(2) | 9/144(8) | 6/136(5) | 6/146(3) | 9/140(8) |
| Micropthalmia | 0/142 | 0/144 | 1/136(1) | 0/146 | 40/140(15) |
| Exencephaly | 0/142 | 0/144 | 1/136(1) | 0/146 | 0/140 |
| Hydrocephalus(lateral) | 0/142 | 0/144 | 1/136(1) | 0/146 | 34/140(12) |
| Hydrocephalus (3rd) | 0/142 | 0/144 | 1/136(1) | 0/146 | 6/140(4) |
| Aortic arch malformed | 0/142 | 1/144(1) | 0/136 | 0/140 | 0/140 |
| Slight hydronephrosis | 44/142(14) | 33/144(14) | 41/136(19) | 38/136(16) | 42/140(18) |
| Total fetuses affected[c] | 46/142 | 46/144 | 49/136 | 47/146 | 86/140 |
| Total litters affected[c] | 15/21 | 19/24 | 20/24 | 16/24 | 23/24 |
| III. Skeleton | | | | | |
| Incomplete ossification of frontal bones | 25/152(9) | 14/156(8) | 11/147(7) | 16/154(9) | 67/157(17) |
| Incomplete ossification of the parietals | 5/152(1) | 3/156(2) | 2/147(2) | 3/154(3) | 11/157(7) |
| Incomplete ossification of intraparietals | 1/152(1) | 0/156 | 1/147(1) | 8/154(4) | 7/157(6) |
| Basioccipital dorsally displaced | 1/152(1) | 1/156(1) | 2/147(1) | 1/154(1) | 3/157(2) |
| Incomplete ossification of maxillary process | 7/152(2) | 2/156(2) | 1/147(1) | 3/154(1) | 1/157(1) |
| Hyoid unossified | 0/152 | 5/156(2) | 0/147 | 0/154 | 5/157(3) |
| Hyoid incompletely ossified | 1/152(1) | 8/156(3) | 0/147 | 1/154(1) | 5/157(3) |
| Enlarged fontanel | 15/152(6) | 6/156(5) | 6/147(5) | 3/154(3) | 39/157(16) |
| Sternebrae incompletely ossified | 3/152(3) | 4/156(2) | 1/147(1) | 10/154(6)* | 8/157(24) |
| Unossified sternebrae | 1/152(1) | 1/156(1) | 3/147(3) | 0/154 | 5/157(5) |
| Vertebral centra lobed | 1/152(1) | 9/156(6) | 10/147(7) | 3/154(2) | 38/157(14) |
| Vertebral centra split | 1/152(1) | 8/156(3) | 3/147(3) | 7/154(3) | 27/157(13) |
| Displaced lumbar vertebrae | 0/152 | 0/156 | 0/147 | 1/154(1) | 1/157(1) |
| Extra ribs | 0/152 | 0/156 | 0/147 | 5/154(2) | 93/157(21) |
| Paw incompletely ossified | 58/152(16) | 88/156(21) | 71/147(22) | 82/154(20) | 96/157(22) |
| Total fetuses affected[c] | 76/152 | 96/156 | 82/147 | 97/154 | 140/157 |
| Total litters affected | 18/21 | 22/24 | 22/24 | 22/24 | 24/24 |

[a] 350 mg/kg hydroxyurea on days 9, 10, and 11 of gestation.
[b] Litters affected.
[c] Includes all abnormalities observed.
* Significantly different than the control $p \leq 0.05$.

# Teratology Study on Rabbits

Fiche Master ID 000000000

Tesh, J.M., et al. (1982). S 276: Effects of oral administration upon pregnancy in the rabbit. An unpublished report (Bayer No. R 2351) prepared by Life Science Research, Essex, England and submitted to Bayer AG, Wuppertal, Germany. Dated December 22, 1982.

Test Chemical:

Disulfoton 97.3%.

Experimental Protocol:

1. Sixty-five sexually mature female New Zealand White rabbits supplied by Morton Rabbits, Essex, England were assigned to the study. The rabbits were 18-24 weeks of age upon receipt and weighed 3.57-4.73 kg at the study initiation. The animals were acclimated to laboratory conditions for three weeks during which time estrus was synchronized by the intravenous administration of 25 IU lutenizing hormone (Pregnyl, Organon).

2. The study was conducted in a room controlled for temperature (16° ± 0.5°C), relative humidity (48 ± 14 percent), and light (14 hours light/10 hours dark). The animals were individually housed in galvanized steel cages with water and Beta Rabbit Standard Diet (Special Diet Services Ltd., Essex, England) available ad libitum. Each female was uniquely identified by an ear-tag.

3. The females wer artificially inseminated with semen pooled from an established fertile colony of male New Zealand White rabbits; immediately after insemination, the females were injected intravenously with 25 IU of luteinizing hormone (Pregnyl, Organon). The day of insemination was designated as day 0 of gestation and the does randomly assigned to four experimental groups. Fourteen does each were assigned to the 0.3 and 1.0 mg/kg dose groups, 22 does to the high dose group, and 25 does to the vehicle control.

4. Daily doses of the test material or the corn oil vehicle were administered by gavage at 0.5 ml/kg on each of days 6 to 18 of gestation.

   Dosing solutions were prepared each day and body weights were determined each day before dosing.

   Initially, the dosages were 0, 0.3, 1.0, and 3.0 mg/kg, but mortality and signs of toxicity influenced the sponsor to reduce the highest dose to 2.0 mg/kg and finally to 1.5 mg/kg. Test material concentrations were determined by GLC in samples of the dosing solutions from the first and last weeks of the dosing period.

5. Maternal signs of toxicity and body weights were recorded daily during the study. On day 29 of gestation the does were sacrificed by intravenous injection of Pentobarbitone sodium (B. Vet. C; Abbott Laboratories) and each examined macroscopically; grossly abnormal tissues were sampled and preserved in "an appropriate fixature."

   The ovaries and attached uterus was removed and weighed, and the following data recorded: number of corpora lutea, number of implantation sites, number of early or late resorption sites, and the number and uterine location of the live and dead fetuses. After removal of the conceptus, the uterus was immersed in a solution of ammonium sulfide to highlight early post-implantation losses.

6. Each fetus and its placenta was weighed, examined for external abnormalities, and the fetus given a subcutaneous lethal injection of phenobarbitone sodium. The thoracic and abdominal cavities and neck of each fetus were opened and examined for soft tissue abnormalities; teh fetuses wer then eviscerated and fixed in "industrial methylated spirit (74° o.p.)". Skeletal development was evaluated for each fetus after processing with a modified Dawson Alizarin technique (Tesh, J.M., 1968. Some effects of aging in spermatozoa on fertility. Ph.D. thesis, Facultyof Veterinary Science, University of Liverpool); the modifications were not described in the final report.

7. Does that aborted were sacrificed by an intravenous injection of phenobarbitone sodium and examined macroscopically; the numbers of corpora lutea and implantation sites were recorded. Where possible, the fetuses were also examined.

8. The multiple t-test or t-test were used to determine statistically significant differences from control in body weight, body weight change, fetal body weight, placental weight, and litter size.

   The Mann-Whitney U-test was used for the corpora lutea count, implantation count, and number of resorptions. Pre- and post-implantation losses were analyzed with Chi-square test, Fischers' Exact Probability test or Mann-Whitney U-test.

RESULTS:

Dosing Solution Analysis: At the first week of dosing, the test material concentrations were 8, 2, and 4 percent below the target concentrations for the low, mid, and high dose groups, respectively. At the last week of dosing, the solutions were 17, 14, and 10 percent below the target concentrations for the low, mid, and high dose groups, respectively. The report suggested that the low values in the last week may be attributable to the loss of the test material to the rubber-lined caps used to seal the sample vials.

Signs of Maternal Toxicity: No signs of toxicity were observed in the low and mid dose groups. In the high-dose groups, signs of toxicity were seen within 4 hours of dosing and, in some cases, persisted for more than 24 hours; the observations included muscular tremor, "unsteadiness/incoordination", and increased respiratory rate.

One low and three control females were found dead or sacrificed in extremis; the diagnosis was middle-ear disease or respiratory tract infection. Compound-induced mortalities were reported in 13/22 does in the high dose group and included 5/5 dosed at 3.0 mg/kg/day, 2/2 dosed at 3.0 and then 2.0 mg/kg, 1/1 at 2.0 mg/kg, and 5/13 at 1.5 mg/kg. Of the nine surviving does, 2 were not pregnant, 1 aborted on day 23 of gestation, and 5 were pregnant; all of these survivors received 1.5 mg/kg throughout the dosing period. One pregnant survivor received 2.0 and then one dose at 1.5 mg/kg for 7 of the 13 dosing days.

In the mid-dose group, 13/14 were pregnant at term and 1 female had aborted. The low dose group contained 12/14 pregnant females and one that was not pregnant. The vehicle control group consisted of 9/14 pregnant does, two that were not pregnant, and one that was removed from the study. Because of the low incidence of viable litters in the vehicle control group, this study included data for the 14 litters of the control group of concurrent study; teh does received distilled water by gavage at 5 ml/kg of body weight.

Maternal Body Weight: No compound related effects were observed in any of the group mean body weights of the does during gestation. Maternal body weights corrected for gravid uterus weight were not significantly different among the groups.

Maternal Necropsy: The necropsy findings in females found dead or sacrificed *in extremis* most frequently included: reduced contents of the gastrointestinal tract and no fecal pellet formation in the high dose group and, in the lower dose groups, lung congestion and consolidation as well as "middle ear filled with thick creamy material".

In the two females that aborted, the mid-dose animal showed no gross abnormalities, while the high-dose female had localized lung congestion, scant contents in the GI tract, and dark fluid contents in the caecum.

Reproductive (litter) Data: No statistically significant differences or compound-related effects were observed in the group mean litter data (Table 1); the parameters included the numbers of corpora lutea, implantations, viable young, and resorptions, as well as implantation losses, fetal body weight, and placental weight. Although the incidence of abortion and total litter loss was within the range from 54 historical studies, the incidence in this study showed a dose-related increase (Table 1).

Fetal Development: Three fetuses from 2 low dose litters had a large number of soft tissue abnormalities that included spina bifida, bilateral hindlimb flexture or malrotation, and rudimentary tail in two fetuses from the same litter, and in the third fetus, short snout, reduced left eye, all lung lobes reduced in size, numerous grooves on liver, liver median lobe adhering to diaphragm, hemorrhagic right ovary, as well as pale spleen and placenta. The foregoing anomalies had not been previously observed in the 54 historical studies used as background data and their occurrance was not clearly related to the test material. One fetus from the supplementary control group ahd encephalocoele, exomphalos, and, among other abnormalities, diaphragmatic hernia. The additional soft tissue anomalies and their historical incidence are shown in Table 2. Compound-related effects were not observed in the fetal soft tissue.

Compound-related effects on skeletal development were apparent when the number of affect litters was expressed as a percentage (Table 3). The observed effects were due to variation in the degree of ossification and indicate fetotoxicity, no teratogenicity.

Spina bifida (open spinal canal with associated parietal flattening) was observed in 2 fetuses from one low dose litter; however despite the rate occurrence of this abnormality, it was not clearly related to the test material. Compound-related effects were not apparent in any of the remaining skeletal anomalies reported.

DISCUSSION:

The study as conducted has several major deficiencies. As a consequence of the high dose lethality, only 5 instead of the recommended 12 pregnant rabbits (Pesticide Assessment Guidelines, USEPA 1982) were available for evaluation of reproductive or teratogenic effects. In addition, only 9 does in the vehicle control group data from another concurrent study were presented in the final report and, despite the does being dosed wiht distilled water at 5 ml/kg/day instead of corn oil at 0.5 ml/kg, the data were within the range of historical data. However, the reason for the low incidence of pregnancy was not apparent nor was it discussed in the report. Although the high dose-level adjustments authorized by the sponsor were appropriate, assigning additional animals to the test group would have been prudent.

The shortcoming of the final report was that no individual data were present for the placental weights and the fetal body weights.

Overall, the sensitivity of this study to detect a reproductive or teratogenic effect of disulfoton was reduced by the small number of pregnancy females in the control and high dose groups; consequently, the study presents weak documentation of negative findings with respect to these possible effects.

CONCLUSIONS:

Under the conditions of this study, S 276 (Disyston) did not produce reproductive or teratogenic effects in the rabbit; however, the study is seriously limited because insufficient numbers of animals were used in the control and high dose groups.

CORE CLASSIFICATION: Supplementary Data.

TABLE 1. Group Mean Litter Data for Does Sacrificed on Day 29 of Gestation

| Dosage (mg/kg/day) | Number of litters | | Abortion and total litter loss (percent) | Corpora lutea count | Implantation | Viable young | | | Resorptions | | | Loss (percent) Implantation | | Foetal weight (g) | Placental weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | M | F | Total | Early | Late | Total | Pre- | Post- | | |
| 0 | 9 | Mean | 0.0 | 10.6 | 6.9 | 2.9 | 3.6 | 5.9 | 0.6 | 0.4 | 1.0 | 34.7 | 14.5 | 46.7 | 7.2 |
| | | S.D. | | 2.4 | 2.8 | 1.6 | 1.8 | 2.9 | 0.7 | 0.7 | 1.0 | | | 1.6 | 0.5 |
| 0[a] | 14 | Mean | 0.0 | 9.7 | 8.5 | 4.0 | 4.0 | 8.0 | 0.2 | 0.3 | 0.5 | 12.5 | 5.9 | 42.4 | 6.4 |
| | | S.D. | | 1.8 | 1.8 | 1.7 | 1.7 | 1.6 | 0.6 | 0.5 | 0.7 | | | 2.0 | 0.3 |
| 0.3 | 12 | Mean | 0.0 | 11.3 | 9.3 | 4.0 | 3.9 | 7.9 | 0.9 | 0.5 | 1.4 | 17.6 | 15.2 | 42.1 | 5.9 |
| | | S.D. | | 2.6 | 1.8 | 1.9 | 1.6 | 1.8 | 1.0 | 0.7 | 1.2 | | | 1.9 | 0.3 |
| 1.0 | 13 | Mean | 7.1 | 9.9 | 8.4 | 3.5 | 3.2 | 6.8 | 0.6 | 1.0 | 1.6 | 15.5 | 19.3 | 43.9 | 6.7 |
| | | S.D. | | 3.3 | 3.3 | 1.6 | 2.0 | 2.7 | 0.8 | 1.0 | 1.3 | | | 2.0 | 0.4 |
| 1.5 | 5 | Mean | 16.7 | 10.8 | 7.6 | 4.4 | 2.6 | 7.0 | 0.2 | 0.4 | 0.6 | 29.6 | 7.9 | 44.8 | 6.3 |
| | | S.D. | | 2.8 | 3.5 | 2.6 | 1.1 | 3.1 | 0.4 | 0.6 | 0.8 | | | 3.7 | 0.4 |
| Historical data from 54 studies | | Overal mean | 5.7 | 11.2 | 9.1 | 4.0 | 4.0 | 8.1 | 0.4 | 0.6 | 1.0 | 19.4 | 10.6 | 41.5 | 5.9[b] |
| | | Recorded ranges | 0.0 | 9.5 | 6.5 | 2.9 | 2.5 | 5.5 | 0.0 | 0.1 | 0.1 | 4.7 | 1.0 | 37.0 | 5.0 |
| | | | 33.3 | 13.5 | 10.9 | 5.1 | 5.5 | 9.6 | 1.1 | 1.4 | 1.6 | 35.7 | 20.5 | 46.9 | 7.1 |

[a] Supplementary control group from a concurrent study; dosed with distilled water at 5 ml/kg body weight.
[b] Placental weight background data derived from 53 studies only.

TABLE 2. Summary of Results of Fetal Soft Tissue Examination: Percent Incidence (Number of Litters)

| Observation | Dose Group (mg/kg/day) | | | | | Historical Data | |
|---|---|---|---|---|---|---|---|
| | 0 | 0[a] | 0.3 | 1.0 | 1.5 | Mean percent incidence | Ranges |
| Number of fetuses (litters) examined | 53(9) | 112(14) | 95(12) | 88(13) | 35(5) | 5370 | 54 studies |
| Hemorrhagic thymus gland | - | - | - | 2.3(1) | - | 0.15 | 0-2.5 |
| Agenesis of median lung lobe | - | - | 1.1(1) | - | 2.9(1) | 0.13 | 0-1.6 |
| Free blood in abdominal cavity | - | | 1.1(1) | - | - | 0.02 | 0-0.7 |
| Abdomen distended | - | - | - | - | 2.9(1) | b | b |
| Gall bladder variants | 11.3(5) | 12.5(9) | 5.3(5) | 17.0(9) | 5.7(2) | 18.73 | 1.0-42.7 |
| Pale area(s) on liver | 1.9(1) | 0.9(1) | 2.1(2) | 3.4(3) | - | 1.14 | 0-4.8 |
| Dark pedunculate discoid mass on liver | 1.9(1) | - | - | - | - | b | b |
| Dark/clear cyst(s) on liver | - | - | 1.1(1) | 1.1(1) | - | 0.04 | 0-1.4 |
| Gas in stomach | - | - | - | - | 2.9(1) | 3.61 | 0-12.5 |
| Stomach contents dark | 1.9(1) | - | - | - | - | 0.02 | 0-1.1 |
| Ovaries hemorrhagic[c] | | 1.8(1) | - | - | | 0.15 | 0-2.4 |
| Testes hemorrhagic unilateral[c] | - | 1.8(1) | - | 2.2(1) | | 0.04 | 0-3.1 |
| Agenesis of left testes[c] | - | - | - | 2.2(1) | - | 0.04 | 0-1.9 |
| Small fetus (<32.0 g) | 9.4(2) | 5.4(5) | 9.5(3) | 9.1(4) | - | 14.0 | 1.0-30.4 |
| Amniotic fluid colored yellow | 1.9(1) | - | - | - | - | b | b |

[a] Supplementary control group from concurrent study; does received distilled water at 5 ml/kg.
[b] Not previously observed.
[c] Expressed as the percentage of male/female fetuses.

TABLE 3. The number and incidence of litters affected with compound-related skeletal anomalies[a]

| Observation | Dose Group (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| | 0 | 0[b] | 0.3 | 1.0 | 1.5 |
| Number of fetuses (litters) examined | 53(9) | 112(14) | 95(12) | 88(13) | 35(5) |
| Incomplete/asymmetric ossification of costal elements of sacral vertebrae | 2(22)[c] | 4(29) | 3(25) | 5(38) | 3(60) |
| Asymmetric pelvis: ilia associated with different sacral vertebrae | 2(22) | 3(21) | 2(17) | 3(23) | 2(40) |
| Small anterior fontanelle | 5(56) | 12(86) | 7(59) | 7(54) | 5(100) |
| Incomplete ossification or absence of hyoid body | 6(67) | 11(78) | 12(100) | 7(54) | 4(80) |

[a] Compiled by this reviewer from the data in the final report.
[b] Supplementary control group from a concurrent gavage study; does received 5 ml/kg/day.
[c] Number of litters (percentage of litters).

003958

Point Mutation in Bacteria

Fiche Master ID 000000000

Hanna, P.J. and Dyer, K.F. (1975). Mutagenicity of Organophosphorus compounds in bacteria and Drosophila. Mutation Res. 28:405-420.

Test Chemical:

Di-Syston 99.3%

Experimental Protocol:

Only the bacterial mutagenicity tests were reviewed since disulfoton was not tested in Drosophila.

Bacterial strains: Ten strains of Salmonella typhimurium LT2 histidine-hisC117, hisG46, hisC207, hisC3076, and hisD3052; Repair: uvrB; Polysaccharide/permeability: gal and rfa. Extensively used S. typhimurium strains were TA 1535, TA 1536, TA 1537, and TA 1538. The Escherichia coli strains used were isogenic E. Tryptophan strains with additional mutations affecting DNA repair (urvA, recA, exrA, and polA). They were identified as WP2, WP2 uvrA, CM 561, CM 571, CM 611, WP 67 and WP 12.

Bacterial Spot Tests: The bacterial strains used in this study had been constructed to accomplish two different objectives, in general; i.e., the Salmonella strains to detect gene (point) mutations and Escherichia coli strains to detect DNA damage. The tests involved addition of a crystal or 5 to 10 l of the test material [140 different organophosphorus compounds (OPs)] to a lawn of the appropriate bacterial strain. For Salmonella testing, 0.1 ml of treatment broth culture was added to 2 ml of molten 0.6 percent agar fortified with 0.5 percent NaCl, 0.02 g/ml L-histidine, and 0.025 g/ml of biotin. These were mixed and poured onto minimal agar plates.[1] After incubation at 37°C for 48 and 72 hr, the number of revertant colonies on the control and test plates was determined and compared. For E. coli, 0.1 ml of the tester strains were spread directly on similar minimal agar plates fortified with 0.75 ug/ml L-tryptophan. After incubation, revertant colonies were assessed on control and test plates.

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[1]Vogel, H.J. and Bonner, D.M. J. Biol. Chem. 21(1956):97-106.

RESULTS:

Bacterial Reverse Mutations: Positive mutagenic responses were induced by Disyston® compound No. 94 in Salmonella strains C 117, G 46, TA 1530, and TA 1535 and E. coli strains WP 2, WP 2uvrA, CM 571, CM 611, WP 67, and WP 12 in the spot assay. This material induced approximately 200 mutant colonies in most strains; however, the authors stated that mutations in the E. coli recA strain CM 561 was much lower than in strains CM 571, CM 611, WP 67, WP 12, WP 2uvrA, and WP 2 (wild type). These data were not presented in the summary of this paper; instead a negative response for this mutant was shown.

DISCUSSION:

The authors concluded that Disyston® of 99.3 percent purity was mutagenic in most of the bacterial strains tested, and that the mutagenicity was unlikely to be due to a contaminating mutagenic compound. From the pattern of response, they considered that it might act similarly to ethylmethanesulfonate, which adds a small moiety (an ethyl group) to DNA causing mutagenic damage not excised by the rec $A^+$ gene product.

CONCLUSIONS:

Our assessment is that the authors' interpretation of a positive mutagenic response is correct from the data presented. Although the data are not quantitative, they provide useful information.

CLASSIFICATION:

Acceptable; however, a quantitative bacterial mutation assay is needed and an autosomal recessive lethal assay should have been performed.

# In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis

Fiche/Master ID 00028625

Simmon, V.F. (1979) "In Vitro" Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides: Report No. EPA-600/1-79-041. (Unpublished study including submitter summary, received Apr. 3, 1980 under 279-2712; prepared by SRI International, submitted by FMC Corp., Philadelphia, Pa.; CDL: 099350-A)

Test Chemical:

Technical Di-Syston, Batch No. 5-08-5110, with unspecified purity.

Experimental Protocol:

Technical Di-Syston was tested for its mutagenic potential in several in vitro microbiological assays and unscheduled DNA synthesis (UDS).

The in vitro assay systems used were the reverse mutation in Salmonella typhurium strains TA1535, TA1537, TA1538, TA98 and TA100 and in Escherichia coli WP2; induction of mitotic recombination in the yeast Saccharomyces cerevisiae D3; relative toxicity assays in DNA repair-proficient and - deficient strains of E. coli (strains W3110 and p3478, respectively) and of Bacillus subtilis (strains H17 and M45, respectively); and unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

1. Reverse mutation assay in Salmonella Typhimurium:

Five strains of S. typhimurium; TA1535, TA1537, TA1538, TA98 and TA100 were used. The tester strains were kept at 4°C on minimal agar plates, supplemented with an excess of biotin and histidine. Ampicillin (25 ug/ml) was added to ensure stable maintenance of plasmid pKM101. The cultures were checked for their genotypic characteristic and for the presence of the plasmid. For each experiment, an inoculum from the stock culture plates was grown overnight at 37°C in nutrient broth. The cultures were then shaken for 3.4 hours. The chemical was tested at the concentrations of 1, 10, 50, 100, 500, and 1000 micrograms per plate with and without metabolic activation according to Ames and McCan (19 ). Negative and positive controls were included. The test was then repeated for the top five concentrations. The chemical was tested for the third time with additional concentrations up to 5000 micrograms per plate with the tester strain TA100.

2. Reverse mutation assay in Escherichia coli WP2:

The tester organism E. coli WP2 is a tryptophan auxotroph (trp) by virtue of a base pair substitution mutation in the tryptophan operon. In addition, WP2 is deficient in the repair of some physically or chemically induced DNA damage.

A procedure similar to Ames Salmonella assay is used to measure the reversion of WP2 to tryptophan independence. However, the minimal agar was supplemented with oxoid nutrient broth (CM67) to provide each plate with the trace of tryptophan required for enhancement of any mutagenic effect of the test chemical. No additional tryptophan is added to the top agar. The chemical was tested at concentrations of 10, 50, 100, 500, 1000 and 5000 micrograms per plate. Negative and positive controls were included.

3. Mitotic recombination assay in Saccharomyces cervisiae D3:

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for at mutation leading to a defective enzyme in the adenine-metabolizing pathway. When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various mutagens. The degree of mutagenicity of a compound or of its metabolite is determined from the number of red-pigmented colonies appearing on the plates.

According to the author, the S. cerevisiae tester strain was stored at -80°C. The tester strain was inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 30°C with aeration.

The in vitro yeast mitotic recombination assay in suspension was conducted as follows. The overnight culture was centrifuged, and the cells were suspended at a concentration of $10^8$ cells/ml in a 67 mM phosphate buffer (pH 7.4). To a sterile test tube the following were added:

- 1.30 ml of the resuspended culture
- 0.50 ml of either the metabolic activation mixture or buffer
- 0.20 ml of a solution of pesticide dissolved in DMSO or 0.20 ml of DMSO alone.

Several doses of the pesticide (up to 5%, w/v or v/v) were tested in each experiment, and appropriate controls were included.

The suspension mixture was incubated at 30°C for 4 hours on a roller drum. The sample was diluted serially in sterile physiological saline, and 0.2-ml aliquots of the $10^{-5}$ and $10^{-3}$ dilutions were spread on tryptone-yeast agar plates; five plates were used for the $10^{-3}$ dilution and three plates were used for the $10^{-5}$ dilution. The plates were incubated for 2 days at 30°C, followed by 2 days at 4°C to enchance the development of the red pigment indicative of adenine-deficient homozygosity. Plates of the $10^{-3}$ dilution were scanned with a dissecting microscope at 10X magnification, and the number of red colonies or red sectors (mitotic recombinants) was recorded. The surviving fraction of organisms was determined from the number of colonies appearing on the plates of the $10^{-5}$ dilution. The number of mitotic recombinants was calculated per $10^{-5}$ survivors.

A positive response in this assay is indicated by a dose-related increase in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per $10^{-5}$ survivors.

4. DNA repair in Escherichia coli W3110/p3478:

Strain p3478 of E. coli is a DNA polymerase deficient (polA) derivation of W3110 and is very sensitive to the effects of chemical and physical agents that react with cellular DNA. The repair assay is based on the finding that when exposed to agents that alter the DNA, bacteria tend to protect themselves by removing the altered DNA segment and then by synthesizing the correct DNA sequence. Thus, their survival is enhanced. The anzymen DNA polymerase is involved in this resynthesizing process. The extent of chemically induced DNA damage can be measured by comparing the relative toxicity (zone of growth inhibition of the two strains. Therefore, if a chemical interacts with DNA, strain p3478 should be more sensitive than strain W3110 to any toxic effect due to this interaction.

For each experiment, an inoculum form frozen stock cultures was grown overnight at 37°C with shaking in nutrient broth consisting of 1% tryptone and 0.5% yeast extract. A 0.1 ml aliquot of this bacterial culture (approximately 3 x $10^8$ cells) was added to 2 ml of nutrient broth containing 0.6% agar. The suspension was mixed and poured onto the surface of a plate containing the same ingredients as the broth plus 2% agar (25 ml). When the top agar had solidified, a

sterile filter disc impregnated with the test substance was placed in the center of the plate. The plates were incubated at 37°C for 16 hours; then the width (diameter) of the zone of inhibition of growth was measured. Several concentrations of the substance were tested. DMSO was used as diluent and as solvent for test chemicals.

The positive control for this assay was 1 phenyl-3,3-dimethyl-triazine. The negative control was chloramphenicol, which should cause equal zones of inhibition in both strains because it is toxic to bacteria but does not kill by interacting with DNA.

5. DNA Repair in Bacillus subtilis H17/M45:

Strain M45 (rec$^-$) of B. subtilis is derived from strain H17 but is deficient in the genetic recombination mechanism necessary to repair DNA damage. Cells deficient in this repair mechanism are killed more easily by chemical mutagens than the wild type cells (rec$^+$). If the chemical is toxic to rec$^-$ cells but at the same concentration is not toxic to rec$^+$ cells, the chemical is assumed to interact with DNA.

To assess the mutagenic potential of Di-Syston in this test, a procedure exactly similar to that used with E. Coli was used.

6. Unscheduled DNA Synthesis Assay:

According to the author WI-38 cells grown in T-25 tissue culture flasks were used for the UDS assays. Replicate cultures of these cells were initiated in Eagle's Basal Medium containing 10% (v/v) fetal calf serum. The cells were grown to confluency and were maintained in medium containing 0.5% serum for 5 to 6 days preceding the DUS assays.* This produced contact-inhibiting cells in synchronous cultures in the $G_0$ phase of the mitotic cycle. To further reduce the possibilfity of incorporationof $^3$H-TdR by anoccasional S-phase cell that might escape the contact-inhibition synchrony and thus obscure measurements of UDS, the cultures were preincubated for 1 hour with $10^{-2}$ M hydroxyurea (HU) before each assay and $10^{-2}$ M HU was added during each subsequent step of the assays.

Dilution of Compounds

Immediately prior to each assay, the pesticide was diluted in an appropriate solvent (ethanol or DMSO) to form a series of concentrations that, when diluted into culture medium,

the appropriate set of test concentrations. To facilitate solubilization or achieve an even suspension of the stock solutions of the compounds in solvent, some of the compounds were sonicated for a brief period of time prior to dilution. The final concentration of solvent was maintained at 1% or less, which we have previously found to be not cytotoxic.

Metabolic Activation

For testing with metabolic activation, a preparation consisting of the 900 x g supernatant of a liver homogenate (250 mg of liver/ml) from adult Swiss-Webster mice was used. To this was added the following cofactors: nicotinamide, 3.05 mg/ml; glucose-6-phosphate, 16.1 mg/ml; $MgCl_2.6H_2O$, 5.08 mg/ml; and NADP, 0.765 mg/ml.

Controls

The positive controls were 4-nitroquinoline-N-oxide (4NQO), a compound that induces UDS in the absence of a metabolic activation system, and dimethylnitrosamine (DMN), a compound that induces UDS in vitro only when an exogenous metabolic activation system is incorporated into the treatment protocol. The negative control was the solvent diluted in culture medium.

Test Procedure

The contact-inhibited WI-38 cells were incubated at 37°C with dilutions of the pesticides and with 1 uCi/ml of $^3$H-TdR (specific activity, 6.7 Ci/mole). For testing in the absence of metabolic activation, the cells were exposed simultaneously to the pesticide and to $^3$H-TdR for 3 hours. For testing with metabolic activation, the cells were incubated together with pesticide, $^3$H-TdR, and the metabolic activation preparation for 1 hour. (The shorter exposure time for metabolic activation testing was used because longer exposures of WI-38 cells to the liver homogenate preparation could be cytotoxic.) In both cases, the cells were then incubated with $^3$H-TdR and HU, but without pesticide, for an additional 3 hours.

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* As a check against the present of mycoplasma, which could incorporate tritiated thymidine ($^3$H-TdR) and thus obscure measurements of UDS, stock cultures were periodically sent to Microbiological Associates, who cultured them on Difco Beef Heart Infusion agar or broth for analysis for the presence of mycoplasma. The results of these analyses were consistently negative.

DNA was extracted from the cells using a modification of the PCA-hydrolysis procedure; one aliquot of the DNA solution was used to measure the DNA content, after the reaction with diphenylamine, and a second aliquot was used for scintillation-counting meausrements of the extent of incorporation of $^{3}$H-TdR. The results were expressed as disintegrations per minute (dpm) of incorporated $^{3}$H-TdR per unit of DNA and were compared with the rate of incorporation of $^{3}$H-TdR into cells exposed to solvent only (negative controls).

We have defined as an acceptable assay one in which the response of the positive control compound is predicted, within the 95% confidence limits, by regressions of average dpm/ug DNA versus average dpm/ug for background. The regressions that follow are based on data that we have acquired in previous testing:

| Type of Testing | Regression* | Sample Size (n) | Correlation Coefficient (r) |
|---|---|---|---|
| Without metabolic activation | $Y_1 = 629+16.42(x)$† | 55 | 0.8066 |
| With metabolic activation | $Y_2 = 212+2.11(x)$† | 25 | 0.8307 |

If the observed average level of incorporation for the positive control compound is outside the 95% confidence limits of the regression, we assume that some variation has occurred in the experimental procedures and the test is repeated.

Metabolic Activation:

The metabolic activation system was prepared according to Ames et al (1975).

References:

B.N. Ames, J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. 31, 347-364 (1975).

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* Regressions over a range of background dpm/ug DNA of 0 to 450.

† $Y_1$ = Average dpm/ug DNA for $10^{-8}$ M 4NQO (positive control).
$Y_2$ = Average dpm/ug DNA for $5 \times 10^{-2}$ M DMN (positive control).
$X$ = Average dpm/ug DNA for background (negative control).
The protocol was taken in its entirety from the original report.

Results:

Results of the reverse mutation assays in S. typhimurium, and E. coli WP2; mitotic recombination assay in S. cerevisia; and DNA repair assays in E. coli W3110 and p3478 and B. subtilis H17 and M45 are presented in tables 1 through 7. The data in these tables indicated that Disulfoton did not exhibit mutagenic activity under testing conditions. However, the data were presented as summary tables, and the number of replicates were not stated.

Results of the unscheduled DNA synthesis in human fibroblasts (WI-38) cells are presented in tables 8 through 11. The data indicated that disulfoton was positive in the UDS assay only in the absence of the metabolic activation. The first test of disulfoton without metabolic activation was statistically positive, yet did not demonstrate a dose-response relationship. The second assay demonstrated dose-related as well as statistically significant increase ($P < 0.01$) increase in UDS.

Discussion and Conclusions:

A positive response in these assays was defined as a reproducible, dose-related increase in the effect being observed. In all the microbial assays, the data were presented as summary tables, and the number of replicates was not stated. It is not known whether the values presented in these tables were in fact an average of a number of replicates or represent single runs, especially with no standard deviations provided. It is difficult to derive a meaningful conclusion with regard to the mutagenic potential of Disulfoton from these data as presented.

The data for the unscheduled DNA synthesis in human fibroblast, indicated that Disulfoton was mutagenic without metabolic activation. On the other hand, it was found in the same report that Demeton, a registered pesticide and a metabolite of Disulfoton, was mutagenic in all the microbial test as well as the USD in human fibroblast assay with and without metabolic activation. Since Demeton is the oxygen analog and a metbolite of Disulfoton, the negative results obtained for Disulfoton in all microbial assays and in the UDS in human fibroblast cells in the presence of metabolic activation could not be explained.

Core Classification:

The in vitro microbial tests are considered as information of supplementary nature, the unscheduled DNA synthesis is acceptable.

003958

# In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis

Fiche/Master ID 00028625

Simmon, V.F. (1979) "In vitro" Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides: Report No. EPA-600/1-79-041, (Unpublished study including submitter summary, recieved April 3, 1980 under 279-2712; prepared by SRI International, submitted by FMC Corp., Philadelphia, Pa.; CDL:099350-A)

Test Chemical:

Technical Di-Syston, Batch No. 5-08-5110, with unspecified purity.

Experimental Protocol:

Technical Di-Syston was tested for its mutagenic potential in several in vitro microbiological assays and unscheduled DNA synthesis (UDS). These in vitro assay systems used were the reverse mutation in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 and in Escherichia coli WP2; induction of mitotic recombination in the yeast Saccharomyces cerevisiae D3; relative toxicity assays in DNA repair-proficient and -deficient strains of E. coli (strains W3110 and p3478, respectively) and of Bacillus subtilis (strains H17 and M45, respectively); and unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

1. Reverse mutation assay in Salmonella typhimurium:

Five strains of S. typhimurium; TA1535, TA1537, TA1538, TA98 and TA100 were used. The tester strains were kept at 4°C on minimal agar plates, supplemented with an excess of biotin and histidine. Ampicillin (25 ug/ml) was added to ensure stable maintenance of plasmid pkM101. The cultures were checked for their genotypic characteristic and for the presence of the plasmid. For each experiment, an inoculum from the stock culture plates was grown overnight at 37°C in nutrient broth. The cultures were then shaken for 3-4 hours. The chemical was tested at the concentration of 1, 10, 50, 100, 500, and 1000 micrograms per plate with and without metabolic activation according to Ames and McCan, (1975). Negative and positive controls were included. The test was then repeated for the top five concentrations. The chemical was tested for the third time with additional concentrations up to 5000 micrograms per plate with tester strain TA100.

2. Reverse mutation assay in Escherichia coli WP2:

The tester organism E. coli WP2 is a tryptophan auxotroph (trp) by virtue of a base-pair substition mutation in the tryptophan operon. In addition, WP2 is deficient in the repair of some physically or chemically induced DNA damage.

A procedure similar to Ames Salmonella assay is used to measure the reversion of WP2 to tryptophan independence. However, the minimal agar was supplemented with Oxoid nutrient broth (CM67) to provide each plate with the trace of tryptophan for enhancement of any mutagenic effect of the test chemical. No additional tryptophan is added to the top agar. The chemical was tested at concentrations of 10, 50, 100, 500, 1000 and 5000 micrograms per plate. Negative and positive controls were included.

3. Mitotic recombination assay in saccharomyces cervisiae D3:

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway. When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various mutagens. The degree of mutagenicity of a compound or of its metabolite is determined from the number of red-pigmented colonies appearing on the plates.

According to the author, the S. cerevisiae tester strain was stored at -80° C. The tester strain was inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 30° C with aeration.

The in vitro yeast mitotic recombination assay in suspension was conducted as follows. The overnight culture was centrifuged, and the cells were resuspended at a concentration of $10^8$ cells/ml in a 67 mM phophate buffer (pH 7.4). To a sterile test tube the following were added:

- 1.30 ml of the resuspended culture
- 0.50 ml of either the metabolic activation mixture or buffer
- 0.20 ml of a solution of pesticide dissolved in DMSO or 0.20 ml of DMSO alone

Several doses of the pesticide (up to 5% w/v or v/v) were tested in each experiment, and appropriate controls were included.

The suspension mixture was incubated at 30° C for 4 hours on a roller drum. The sample was diluted serially in sterile physiological saline, and 0.2-ml aliquots of the $10^{-3}$ and $10^{-3}$ dilutions were spread on tryptone-yeast agar plates; five plates were used for the $10^{-3}$ dilution and three plates were used for the $10^{-5}$ dilution. The plates were incubated for 2 days at 30° C, followed by two days at 4° C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates of the $10^{-3}$ dilution were scanned with a dissecting microscope at 10X magnification, and the number of red colonies or red sectors (mitotic recombinations) was recorded. The surviving fraction of organisms was determined from the number of colonies appearing on the plates of the $10^{-5}$ dilution. The number of mitotic recombinations was calculated per $10^5$ survivors.

A positive response in this assay is indicated by a dose-related increase in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants recombinants per $10^5$ survivors.

4. DNA repair in Escherichia coli W3110/p3478:

Strain p3478 of E. coli is a DNA polymerase deficient (polA) derivation of W3110 and is very sensitive to the effects of chemical and physical agents that react with cellular DNA. The repair assay is based on the finding that when exposed to agents that alter the DNA, bacteria tend to protect themselves by removing the altered DNA segment and then by synthesizing the correct DNA sequence. Thus, their survival is enhanced. The enzyme DNA polymerase is involved in this resynthesizing process. The extent of chemically induced DNA damage can be measured by comparing the relative toxixcity (zone of growth inhibition) of the two strains. Therefore, if a chemical interacts with DNA, strain p3478 should be more sensitive than strain W3110 to any toxic effect due to this interaction.

For each experiment, an inoculum from stock cultures was grown overnight at 37° C with shaking in nutrient broth consisting of 1% tryptone and 0.5% yeast extract. A 0.1-ml aliquot of this bacterial culture (approximately 3 X $10^8$ cells) was added to 2 ml of nutrient broth containing 0.6% agar. The suspension was mixed and poured onto the suface of a plate containing the same ingredients as the broth plus 2% agar (25 ml). When the top agar had solidified, a sterile filter disc impregnated with the test substance was placed in the center of the plate. The plates were incubated at 37° C for 16 hours; then the width (diameter) of the zone of inhibition of growth was measured. Several concentrations of the substance were tested. DMSO was used as diluent and as solvent for test chemicals.

The positive control for this assay was 1-phenyl-3,3-dimethyltriazine. The negative control was chloramphenicol, which should cause equal zones of inhibition in both strains because it is toxic to bacteria but does not kill by interacting with DNA.

5. DNA repair in Bacillus subtilis H17/M45:

Strain M45 (rec$^-$) of B. subtilis is derived from the strain H17 but is deficient in the genetic recombination mechanism necessary to repair DNA damage. Cells deficient in this repair mechanism are killed more easily by chemical mutagens than the wild type cells (rec$^+$). If the chemical is toxic to rec$^-$ cells but at the same concentration is not toxic to rec$^+$ cells, the chemical is assumed to interact with DNA.

To assess the mutagenic potential of Di-Syston in this test, a procedure exactly similar to that of used with E. coli was used.

6. Unscheduled DNA Synthesis Assay:

According to the author, WI-38 cells grown in T-25 tissue culture flasks were used for the UDS assays. Replicate cultures of these cells were initiated in Eagle's Basal Medium containing 10% (v/v) fetal calf serum. The cells were grown to confluency and were maintained in medium containing 0.5% serum for 5 to 6 days preceding the UDS assays.* This produced contact-inhibited cells in synchronous cultures in the $G_0$ phase of the mitotic cycle. To further reduce the posibility of incorporation of $^3$H-TdR by an occasional S-phase cell that might escape the contact-inhibition synchrony and thus obscure measurements of UDS, the cultures were preincubated for 1 hour with $10^{-2}$ M hydroxyurea (HU) before each assay and $10^{-2}$ M HU was added during each subsequent step of the assays.

Dilution of Compounds

Immediately prior to each assay, the pesticide was diluted in an appropriate solvent (ethanol or DMSO) to form a series of concentrations that, when diluted into culture medium, yeilded the appropriate set of test concentrations. To facilitate solubilization or achieve an even suspension

* As a check against the presence of mycoplasma, which could incorporate tritiated thymidine ($^3$H-TdR) and thus obscure measurements of UDS, stock cultures were periodically sent to Micrbiological Associates, who cultured them on Difco beef heart Infusion agar or broth for analysis for the presence of mycoplasma. The results of these analyses were consistently negative.

of the stock solutions of the compounds in solvent, some of the compounds were sonicated for a brief period of time prior to dilution. The final concentation of solvent was maintained at 1% or less, which we have previously found to be not cytotoxic.

Metabolic Activation

For testing with metabolic activation, a preparation consisting of the 9000 x g supernatant of a liver homogenate (250 mg of liver/ml) from adult Swiss-Webster mice was used. To this was added the following cofactors: nicotinamide, 3.05 mg/ml; glucose-6-phosphate, 16.1 mg/ml; $MgCl_2.6H_2O$, 5.08 mg/ml; and NADP, 0.765 mg/ml.

Controls

The positive controls were 4-nitroquinoline-N-oxide (4NQO), a compound that induces UDS in the absence of a metabolic activation system, and dimethylnitrosamine (DMN), a compound that induces UDS in vitro only when an exogenous metabolic activation system is incorporated into the treatment protocol. The negative control was the solvent diluted in culture medium.

Test Procedure

The contact-inhibited WI-38 cells were incubated at 37° C with dilutions of the pesticides and with 1 uCi/ml of $^3$H-TdR (specific activity, 6.7Ci/mmole). For testing in the absence of metabolic activation, the cells were exposed simultaneously to the pesticide and to $^3$H-TdR for the cells were exposed simultaneously to the pesticide and to $^3$H-TdR for three hours. For testing with metabolic activation, the cells were incubated together with pesticide, $^3$H-TdR and the metabolic activation preparation for 1 hour. (The shorter exposure time for metabolic activation testing was used because longer exposures of WI-38 cells to the liver homogenate preparation could be cytotoxic.) In both cases, the cells were than incubated with $^3$H-TdR and HU, but without pesticide, for an additional 3 hours.

DNA was extracted from the cells using a modification of the PCA-hydrolysis procedure one aliquot of the DNA solution was used to measure the DNA content, after the reaction with diphenylamine, and a second aliquot was used for scintillation-counting measurements of the extent of incorporation of $^3$H-TdR. The results were expressed as

disintegrations per minute (dpm) of incorporated $^3$H-TdR per unit of DNA and were compared with the rate of incorporation of $^3$H-TdR into cells exposed to solvent only (negative controls).

We have defined as an acceptable assay one in which the response of the positive control compound is predicted, within the 95% confidence limits, by regressions of average dpm/ug DNA versus average dpm/ug for background. The regressions that follow are based on data that we have acquired in previous testing:

| Type of Testing | Regression* | Sample Size (n) | Correlation Coefficient (r) |
|---|---|---|---|
| Without metabolic activation | $Y_1 = 629 + 16.42(X)$† | 55 | 0.8066 |
| With metabolic activation | $Y_2 = 212 + 2.11(X)$† | 25 | 0.8307 |

If the observed average level of incorporation for the positive control compound is outside the 95% confidence limits of the regression, we assume that some variation has occurred in the experimental procedures and the test is repeated.

Metabolic Activation:

The metabolic activation system was prepared according to Ames et al. (1975).

References:

B. N. Ames, J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutation Res. 31, 347-364 (1975).

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* Regressions over a range of background dpm/ug DNA of 0 to 450.

† $Y_1$ = Average dpm/ug DNA for $10^{-3}$ M 4NQO (positive control)
$Y_2$ = Average dpm/ug DNA for 5 X $10^{-2}$ M DMN (positive control)
$X$ = Average dpm/ug DNA for background (negative control)
The protocol was taken in its entirety from the original report.

Results:

Results of the reverse mutation assays in S. typhimurium, and E. coli WP2; mitotic recombination assay in S. cerevisiae; and DNA repair assays in E. coli W3110 and p3478 and B. subtilis H17 and M45 are presented in tables 1 through 7. The data in these tables indicated that Disulfoton did not exhibit mutagenic activity under testing conditions. However, the data were presented as summary tables, and the number of replicates were not stated.

Results of the unscheduled DNA synthesis in human fibroblasts (WI-38) cells are presented in tables 8 through 11. The data indicated that Disulfoton was positive in the UDS assay only in the absence of the metabolic activation. The first test of disulfoton without metabolic activation was statistically positive, yet did not demonstrate a dose response relationship. The second assay demonstrated dose-related as well as statistically significant increase ($p<0.01$) increase in UDS.

Discussions and Conclusions:

A positive response in these assays was defined as a reproducible, dose-related increase in the effect being observed. In all the microbial assays, the data were presented as summary tables, and the number of replicates was not stated. It is not known whether the values presented in these tables were in fact an average of a number of replicates or represent single runs, especially with no standard deviations provided. It is difficult to derive a meaningful conclusion with regard to the mutagenic potential of Disulfoton from these data as presented.

The data for the unscheduled DNA synthesis, in human fibroblast, indicated that Disulfoton was mutagenic without metabolic activation. On the other hand, it was found in the same report that Demeton, a registered pesticide and a metabolic of Disulfoton, was mutagenic in all the microbial test as well as the USD in human fibroblasts assay with and without metabolic activation.. Since Demeton is the oxygen analog and a metabolite of Disulfoton, the negative results obtained for Disulfoton, the negative results obtained for Disulfoton in all microbial assays and in the UDS in human fibroblast cells in the presence of metabolic activation could not be explained.

Core Classification:

The in vitro microbial tests are considered as information of supplementary nature, the unscheduled DNA synthesis is acceptable.

Table 1

In Vitro Assays With Salmonella Typhimurium

of Disulfoton

Experiment 1

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative Control | - | | 14 | 8 | 7 | 22 | 163 |
| | + | | 10 | 15 | 27 | 44 | 176 |
| Positive Controls | | | | | | | |
| 2-Anthramine | - | 20 | | | 39 | 27 | |
| | + | 20 | | 200 | 2880 | 3840 | |
| -Propiolactone | - | 50 | 1980 | | | | 2520 |
| Disulfoton | - | 1 | 12 | 13 | 10 | 22 | 116 |
| | - | 10 | 16 | 7 | 15 | 19 | 136 |
| | - | 50 | 19 | 9 | 7 | 18 | 112 |
| | - | 100 | 17 | 12 | 11 | 15 | 109 |
| | - | 500 | 18 | 6 | 10 | 23 | 146 |
| | - | 1000 | 23 | 6 | 12 | 26 | 133 |
| | + | 1 | 9 | 13 | 17 | 32 | 121 |
| | + | 10 | 10 | 15 | 20 | 34 | 137 |
| | + | 50 | 7 | 9 | 15 | 41 | 121 |
| | + | 100 | 10 | 10 | 16 | 22 | 128 |
| | + | 500 | 22 | 9 | 14 | 34 | 135 |
| | + | 1000 | 23 | 13 | 17 | 29 | 152 |

Table 2

In Vitro Assays With Salmonella Typhimurium

of Disulfoton

Experiment 2

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative Control | - | | 29 | 15 | 30 | 44 | 226 |
| | + | | 25 | 18 | 33 | 51 | 251 |
| Positive Controls | | | | | | | |
| AF2 | - | 0.5 | | | | 190 | 720 |
| 2-Anthramine | - | 20 | 1330 | 700 | 3800 | 4940 | 8000 |
| | - | 10 | 25 | 17 | 31 | 24 | 178 |
| | - | 50 | 29 | 15 | 30 | 49 | 196 |
| | - | 100 | 30 | 16 | 33 | 32 | 192 |
| | - | 500 | 25 | 18 | 29 | 51 | 228 |
| | - | 1000 | 44 | 22 | 39 | 50 | 206 |
| | | 5000 | 57 | 18 | 14 | 50 | 224 |
| | + | 10 | 35 | 17 | 32 | 55 | 142 |
| | + | 50 | 21 | 17 | 37 | 53 | 146 |
| | + | 100 | 21 | 21 | 42 | 55 | 214 |
| | + | 500 | 24 | 16 | 33 | 53 | 258 |
| | + | 1000 | 38 | 17 | 36 | 45 | 172 |
| | | 5000 | 48 | 22 | 34 | 32 | 202 |

Table 3

In Vitro With Salmonella Typhimurium

of Disulfoton

Experiment 3

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA100 |
|---|---|---|---|
| Negative Control | - | | 126 |
| | + | | 150 |
| Positive Control 2-Anthramine | + | 2.5 | 558 |
| Disulfoton | - | 10 | 143 |
| | - | 50 | 125 |
| | - | 100 | 148 |
| | - | 500 | 130 |
| | - | 1000 | 138 |
| | - | 2000 | 143 |
| | - | 3000 | 145 |
| | - | 4000 | 150 |
| | - | 5000 | 164 |
| | - | | |
| | + | 10 | 143 |
| | + | 50 | 124 |
| | + | 100 | 132 |
| | + | 500 | 129 |
| | + | 1000 | 149 |
| | + | 2000 | 148 |
| | + | 3000 | 155 |
| | + | 4000 | 148 |
| | + | 5000 | 141 |

Table 4

In Vitro Assay With Escherichia Coli WP2

Experiment 1

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Tryptophan Revertants per Plate |
|---|---|---|---|
| Negative Control | - | | 26 |
| | + | | 49 |
| Positive Controls | | | |
| 2-Anthramine | + | 2.5 | |
| -Propiolactone | - | 10 | 91 |
| N-Methyl-N'-nitro-N-nitrosoguanidine | - | 2.0 | |
| AF2 | - | 0.1 | |
| Disulfoton | - | 1 | 21 |
| | - | 10 | 23 |
| | - | 50 | 21 |
| | - | 100 | 24 |
| | - | 500 | 17 |
| | - | 1000 | 28 |
| | + | 1 | 31 |
| | + | 10 | 21 |
| | + | 50 | 41 |
| | + | 100 | 27 |
| | + | 500 | 26 |
| | + | 1000 | 22 |

Table 5

In Vitro Assay With Escherichia Coli WP2

Experiment 2

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Tryptophan Revertants per Plate |
|---|---|---|---|
| Negative Control | - | | 31 |
| | + | | 37 |
| Positive Controls | | | |
| N-Methyl-N'-nitro-N-nitrosoguanidine | - | 2.0 | |
| AF2 | - | 0.1 | 1044 |
| 2-Anthramine | - | 2.5 | |
| | + | 2.5 | |
| Disulfoton | - | 10 | 34 |
| | - | 50 | 35 |
| | - | 100 | 32 |
| | - | 500 | 29 |
| | - | 1000 | 45 |
| | - | 5000 | 24 |
| | - | 10000 | NT |
| | + | 10 | 33 |
| | + | 50 | 32 |
| | + | 100 | 31 |
| | + | 500 | 38 |
| | + | 1000 | 45 |
| | + | 5000 | 42 |
| | + | 10000 | NT |

In Vitro Assays with Saccharomyces Cerevisiae
Disulfoton

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors: Cells per ml (x $10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml (x $10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| | | Experiment 1 | | | | |
| Negative Control | - | | 7.8 | 100 | 2 | 2.6 |
| | + | | 7.0 | 100 | 4 | 5.7 |
| Positive Control 1,2,3,4-Diepoxybutane | - | 0.04 | 6.6 | 85 | 1602 | 2427 |
| | + | 0.04 | 5.6 | 80 | 1365 | 2437 |
| Disulfoton | - | 0.1 | 8.3 | 106 | 6 | 7.2 |
| | - | 0.5 | 7.2 | 92 | 3 | 4.2 |
| | - | 1.0 | 9.4 | 120 | 12 | 12.8 |
| | - | 5.0 | 9.3 | 119 | 5 | 5.4 |
| | + | 0.1 | 8.0 | 114 | 3 | 4.2 |
| | + | 0.5 | 8.1 | 116 | 5 | 6.2 |
| | + | 1.0 | 9.4 | 134 | 8 | 8.5 |
| | + | 5.0 | 7.9 | 113 | 14 | 17.7 |
| | | Experiment 2 | | | | |
| Negative Control | - | | 4.8 | 100 | 3 | 6.3 |
| | + | | 4.7 | 100 | 3 | 6.4 |
| Positive Control 1,2,3,4-Diepoxybutane | - | 0.04 | 3.4 | 71 | 745 | 2191 |
| | + | 0.04 | 3.5 | 74 | 683 | 1951 |
| Disulfoton | - | 1.0 | 3.5 | 73 | 2 | 5.7 |
| | - | 2.0 | 4.3 | 90 | 4 | 9.3 |
| | - | 4.0 | 4.5 | 94 | 4 | 8.9 |
| | - | 5.0 | 5.4 | 113 | 3 | 5.6 |
| | + | 1.0 | 3.9 | 83 | 3 | 7.7 |
| | + | 2.0 | 4.3 | 91 | 8 | 18.6 |
| | + | 4.0 | 4.7 | 100 | 5 | 10.6 |
| | + | 5.0 | 4.6 | 98 | 9 | 19.6 |

Table 6 Cont'd.

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| | | Experiment 3 | | | | |
| Negative Control | - | | 8.2 | 100 | 2 | 2.4 |
| | + | | 8.1 | 100 | 3 | 3.7 |
| Positive Control 1,2,3,4-Diepoxy-butane | - | 0.04 | 6.1 | 74 | 868 | 1423 |
| | + | 0.04 | 5.7 | 70 | 945 | 1658 |
| Disulfoton | - | 1.0 | 9.1 | 111 | 2 | 2.2 |
| | - | 2.0 | 7.3 | 89 | 3 | 4.1 |
| | - | 4.0 | 5.9 | 72 | 9 | 15.3 |
| | - | 5.0 | 8.5 | 104 | 4 | 4.7 |
| | + | 1.0 | 8.6 | 106 | 4 | 4.7 |
| | + | 2.0 | 7.7 | 95 | 7 | 9.1 |
| | + | 4.0 | 7.9 | 98 | 2 | 2.5 |
| | + | 5.0 | 7.8 | 96 | 6 | 7.7 |

Table 7

Differential Toxicity of Repair-Proficient and -Deficient Microorganisms

| Compound | mg of Compound in 10 uL of DMSO Applied to Disc | Diameter of Zone of Inhibition (mm)* | | | |
|---|---|---|---|---|---|
| | | B. subtilis | | E. coli | |
| | | H17 | M45 | W3110 | p3478 |
| Negative Control Chloramphenicol | 20 ug | 38 | 40 | 40 | 40 |
| Positive Control 1-phenyl-3,3-dimethyl-triazine | 2 | 12 | 20 | 13 | 20 |
| Disulfoton | 0.01 | 6 | 6 | 6 | 6 |
| | 0.10 | 6 | 6 | 6 | 6 |
| | 1.0 | 6 | 6 | 6 | 6 |
| | 5.0 | 6 | 6 | 6 | 6 |

* The diameter of the disc was 6mm.

Table 8

Unscheduled DNA Synthesis Assay of Disulfoton
Without Metabolic Activation
(dpm/ug DNA)

| Sample | Concentration of Compounds Tested: Disulfoton (ug/ml) 0* | 0.1 | 1.0 | 10 | 100† | 1000† | 4NQO (M) $10^{-5}$ |
|---|---|---|---|---|---|---|---|
| 1 | 67 | 40 | 33 | 23 | 64 | 92 | 4367 |
| 2 | 58 | 32 | 27 | 31 | 43 | 76 | 3593 |
| 3 | --§ | 40 | 38 | 43 | 59 | 111 | 2267 |
| 4 | 78 | 37 | 58 | 32 | 73 | 106 | 3039 |
| 5 | 71 | 26 | 30 | 36 | 68 | 109 | 2960 |
| 6 | 57 | 27 | 51 | 31 | 65 | 107 | 4142 |
| Mean | 66 | 34 | 39 | 33 | 62 | 100 | 3395 |
| SD | 9 | 6 | 12 | 7 | 11 | 14 | 791 |
| SE | 4 | 3 | 5 | 3 | 4 | 6 | 323 |

* Negative control and compound solvent, 0.5% ETOH.

† Precipitates observed at 100 and 1000 ug/ml.

§ Sample lost.

Table 9

Repeat Unscheduled DNA Synthesis Assay of Disulfoton
Without Metabolic Activation
(dpm/ug DNA)

| | Concentration of Compounds Tested | | | | | | |
|---|---|---|---|---|---|---|---|
| | Disulfoton (ug/ml) | | | | | | 4NQO (M) |
| Sample | 0* | 250† | 500† | 1000† | 2000† | 4000 | $10^{-5}$ |
| 1 | 162 | 173 | 222 | 272 | 361 | 271 | 2293 |
| 2 | 158 | 142 | 200 | 294 | 258 | 335 | 2200 |
| 3 | 132 | 149 | 180 | 281 | 249 | 267 | 2604 |
| 4 | 109 | 163 | 214 | 209 | 267 | 272 | 2681 |
| 5 | 202 | 179 | 188 | 223 | 305 | 291 | 2066 |
| 6 | 160 | 202 | 218 | 237 | 397 | 248 | 2316 |
| Mean | 154 | 168 | 203 | 253 | 302 | 281 | 2360 |
| SD | 31 | 22 | 17 | 35 | 53 | 30 | 237 |
| SE | 13 | 9 | 7 | 14 | 22 | 12 | 97 |

* Negative control and compound solvent, 0.5% DMSO.

† Precipitates observed at all concentrations.

Table 10

003958

Unscheduled DNA Synthesis Assay of Disulfoton
With Metabolic Activation
(dpm/ug DNA)

| | Concentration of Compounds Tested | | | | | | |
|---|---|---|---|---|---|---|---|
| | Disulfoton (ug/ml) | | | | | | DMN (M) |
| Sample | 0* | 0.1 | 1.0 | 10 | 100† | 1000† | $5 \times 10^{-2}$ |
| 1 | 223 | 188 | 163 | 146 | 211 | 163 | 1163 |
| 2 | 191 | 190 | 163 | 152 | 224 | 159 | 969 |
| 3 | 209 | 208 | 175 | 202 | 202 | 214 | 1023 |
| 4 | 204 | 209 | 77 | 164 | 173 | 202 | 956 |
| 5 | 181 | 218 | 165 | 165 | 220 | 227 | 1019 |
| 6 | 194 | 166 | 153 | 206 | 207 | 190 | 1008 |
| Mean | 200 | 197 | 149 | 172 | 206 | 193 | 1023 |
| SD | 15 | 19 | 36 | 25 | 18 | 27 | 74 |
| SE | 6 | 8 | 15 | 10 | 7 | 11 | 30 |

* Negative control and compound solvent, 0.5% DMSO.

† Precipitates observed at 100 and 1000 ug/ml.

Table 11

Repeat Unscheduled DNA Synthesis Assay of Disulfoton
With Metabolic Activation
(dpm/ug DNA)

| | Concentration of Compounds Tested | | | | | | |
|---|---|---|---|---|---|---|---|
| | Disulfoton (ug/ml) | | | | | | DMN (M) |
| Sample | 0* | 250† | 500† | 1000† | 2000† | 4000† | 5 x $10^{-2}$ |
| 1 | --§ | 122 | 127 | 113 | 116 | 127 | 383 |
| 2 | 187 | 171 | 118 | 121 | 110 | 111 | 242 |
| 3 | 164 | 119 | 110 | 62 | 86 | 108 | 263 |
| 4 | 137 | 118 | 169 | 150 | 106 | 121 | 289 |
| 5 | 144 | 180 | 183 | 110 | 116 | 93 | 297 |
| 6 | 143 | 124 | 165 | 137 | 111 | 115 | 372 |
| Mean | 155 | 139 | 146 | 115 | 108 | 112 | 308 |
| SD | 21 | 29 | 31 | 30 | 11 | 12 | 58 |
| SE | 9 | 12 | 13 | 12 | 5 | 5 | 24 |

* Negative control and compound solvent, 0.5% DMSO.

† Precipitates observed at all concentrations.

§ Sample lost

Some tables and parts of the experimental protocol were taken from the original report without change.

Dominant Lethal Study on Male Mouse

Fiche/Master ID 000000000

Herbold, B. (1980) Dominant Lethal Study on Male Mouse to Evaluate S-276 for Mutagenic Potential. (Unpublished Study, Bayer AG, Institute for Toxicology, Report No. 9440).

Test Chemical:

Technical Di-Syston 94.9%.

Experimental Protocol:

A preliminary evaluation of the toxicity of disulfoton was conducted to determine the dose to be used in the main experiment. Single doses of 3 or 5 mg/kg were administered by gavage to NMRI/ORIG Kislegg mice. The authors reported that the effects observed were mild. No further details regarding the effects were given. The test substance was administered in aqueous Cremphor (0.5%) emulsion.

A single dose of 5 mg disulfoton per kg body weight was given to the treatment group. The treated control mice received an equal volume of emulsion without disulfoton. Each group contained 50 males which were placed with virgin untreated female mice for a four day mating period. At the end of the first mating period females were replaced. There were 12 consecutive matings covering the 48 days following dosing.

Fourteen days after the mating females were sacrificed and examined. Corpora lutea and implantation sites were counted. The number of viable implants and dead implants (sum of decidiomata, resorptions and dead embryos) were also recorded.

Total and dead implant data were transformed using square roots, and the angular transformation was used for the ratio of dead to total implants. These transformed data were subjected to analysis of variance, Dunett's or tukey's tests where appropriate to determine statistical significance of differences. Frequency distributions for the parameters measured were also analyzed with the Komogrov-Smirnov test.

Results:

The author reported no effect on the fertility index (number of pregnant females per number mated multiplied by 100) or post implantation losses (sum of deciduomata, resorptions,

and dead embryos divided by the total number of implants which is then by 100). The reported results (overall group means and ranges from 12 mating periods) are summarized as follows:

| Parameter | Control | Treated |
|---|---|---|
| Fertility (%) | 73.7 (64-82) | 73.3 (60-85.7) |
| Corpora lutea* | 10.9 (10.2-11.4) | 11.0 (10.3-11.5) |
| Implantations* | 10.5 (9.5-11.1) | 10.7 (10.1-11.3) |
| Pre-implantation losses* | 0.36 (0.08-0.76) | 0.31 (0.16-0.62) |
| Viable Implants* | 9.9 (9.2-10.6) | 10.0 (9.3-10.5) |
| Dead Implants* | 0.60 (0.25-1.27) | 0.68 (0.27-1.57) |

* Per female

Conclusions:

The data presented in this report support the author's conclusion that the test chemical is not mutagenic under the test conditions. However, the toxicological significance of the results is limited since only one dose level was used and no positive control data were presented.

Core Classification:

Unacceptable.

This study was originally reviewed by R. Gardner and further evaluated by G. Ghali for re-registration purposes.

# Micronucleus Test in Mice

Fiche Master ID 000000000

Herbold, B. (1981). S 276, Disulfoton, Thio-Demeton® DISYSTON-active ingredient. Micronucleus test on the mouse to evaluate for mutagenic effect. Report No. 10451, prepared by Bayer AB Institute of Toxicology for Mobay Chemical Corporation. Dated December 23, 1981.

Test Chemical:

The test material was identified as S 276, batch 79/R/255/40 to 50 percent purity.

Experimental Protocol:

Animal Phase: The test species was identified as strain Bor:NMRI(SPF Han) and included male and female mice which weighed between 25-34 g at the start of the study. The mice which were between 8 and 12 weeks old, were separated by their sex and test groups and caged in groups (a maximum of 3) in Makrolon type I cages. Both cage and picric acid markings were used to identify the animals. The food, identified as "Altromin 1324, Altromin GmbH, Lage," and tapwater were given ad libitum.

Study Design: Table 1 describes the groups and their dosing regimen.

The doses of the test material, Disyston® Technical, were based on a range finding experiment that used administration of two doses at 10, 5, and 2.5 mg/kg, respectively in five animals. Two p.o. doses of 5 mg/kg of the test material were tolerated without symptoms, and two doses of the 50 percent food-test material pre-mix were tolerated without symptoms at 6- and 12 mg/kg, respectively.

Slide Preparation: The procedure for preparing and staining bone marrow cells was that described by Schmid.[1]

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[1]Schmid, W. 1975. Mutation Research 31:9-15.

TABLE 1

| Group Designation | Size[a] | Sex | | Dose[b] (mg/kg) | Route of Application |
|---|---|---|---|---|---|
| | | Male | Female | | |
| Vehicle Control | 10 | 5 | 5 | 0 | p.o. |
| S 276 (L) | 10 | 5 | 5 | 6 | p.o. |
| S 276 (H) | 10 | 5 | 5 | 12 | p.o. |
| Trenimon (positive control) | 10 | 5 | 5 | 0.125 | i.p. |

[a]Randomly assigned according to a "PH-DB" plan.

[b]Dosing at the level indicated was administered p.o. twice at 24 hour intervals, and the animals were sacrificed by decapitation and their femoral marrow taken 6 hours after second dosing. The application volume was 10 ml/kg for all p.o. groups, using 0.5 percent Cremophor as the suspension medium and also as the vehicle control.

Evaluation of Data: Slides of each animal were randomly scanned and 1000 polychromatic erythrocytes (PCEs) were scored to establish the incidence of micronuclei. Also the ratio of PCEs to normochromatic erythrocytes (NCEs) was determined to identify pathology (if present) not associated with the test material in each animal, and to assess general effects, e.g., erythropoiesis, caused by the test material. If the NCE to PCE ratio exceeded 3:1 in a single animal, for example, the animal could be excluded from further data analyses on the assumption that its pathology was not compound-related.

Statistics: The NEMENITY non-parametric ranking test was applied to the data, using $p < 0.05$ as a statistically significant result. The positive control was excluded from this evaluation.

RESULTS:

Dose Tolerance: Oral doses of S 276 up to 12 mg/kg given twice (separated by 24 hr) had no apparent effect on behavior, mortality, appearance or motoric functions. No compound-induced mortalities occurred.

Evaluation of Marrow Cells: The mean incidence of micronuclei in PCEs of the control animal marrow cells was 1.9 per thousand in the combined negative control; for this computation it was 2.2 per thousand in the males and 1.6 percent thousand in the females. In this control group the mean ratio of PCEs to NCEs was 1000 to 473. Mice treated with S 276 for 2 times at 6 and 12 mg/kg averaged 2.0 and 1.6 micronuclei per 1000 PCEs, respectively, with the incidence of micronuclei being similar in both sexes. Mice treated with the positive control, Trenimon, twice by the i.p. route at a dose of 0.125 mg/kg, averaged 58 micronuclei per 1000 PCEs, with no significant differences between the sexes. Internal all groups showed no statistically significant differen and there was no statistical difference between the vehicle trol and S 276-treated animals.

Trenimon treatment caused bone marrow depression since the ratio of PCEs CEs was 1000 to 1291 versus 1000 to 473 in the vehicle control p. Although th mean values of groups treated with S 276 had PCE CE ratios which were within normal ranges, there were three animals whose PCE to NCE ratios fell within normal ranges, there were three animals whose PCE to NCE ratios feel within the range exhibited by the positive control mice. Two females in the 6 mg/kg dosage group had PCE:NCE ratios of 1000 to 1068 and 1000 to 1060, spectively. One female in the 12 mg/kg dosage had a PCE to NCE ratio of 1000 to 1252. However, none of these mice had elevated numbers of micronuclei compared with their concurrent control groups.

DISCUSSIC

The auth concluded that S 276, Disyston® Technical, did not induce an *in vivo* increase in micronuclei at doses up to 2 x 12 mg/kg under the conditions of the study and that the ratio of PCEs to NCEs "did change to a biologically relevant degree" compared to the ne tive control. However, the positive control, Trenimon, inhibited rythropoiesis and induced micronuclei at a level which was stati cally significant.

Our asses nt is that the author's conclusions from the data were gene ly correct, with the notation that 3 females in S 276 treatment roups had unfavorable PCE to NCE ratios (see results) which indi ted an inhibition of erythropoiesis.

Two import t criticisms of the study include an insufficient dosage and insufficient sampling for micronuclei. The treatment with the test material should have been given at a dosage approximately 80 percent of the 7 day $LD_{50}$ to insure the maximum

opportunity to induce chromosome breakage. Since mammalian erythrocytes do not contain nuclei (they are expelled 8-12 hour after the last mitosis), sampling less than 8-12 hours after treatment is ineffective in the detection of micronuclei[2]. We suggest, therefore, that the testing procedure was not sufficiently rigorous to detect a clastogenic effect produced by the test material.

CONCLUSIONS:

The test material, S 276, containing 50 percent Disyston®, was not clastogenic at doses of 2 x 6 mg/kg or 2 x 12 mg/kg under the conditions of the assay. However, the assay dosage was insufficient and sampling periods were not frequent enough or at intervals appropriate for detecting some micronuclei which could have been produced.

CLASSIFICATION: Unacceptable.

The dosage was insufficient and the sampling periods were not adequate to document a negative response.

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[2]Heddle, J.A., Salamone, M.F. 1981. The micronucleus assay. In Vivo in Stich, H.F. and San R.H.C., eds. Short-term test for chemical carcinogens, Springer-Verlag N.Y./Heidelberg/Berlin.

Sister Chromatid Exchange

Fiche Master ID 000000000

Chen, H.H., Hsueh, J.L., Sirianni, S.R., and Huang, C.C. (1981). Induction of sister chromatid exchanges and cell cycle delay in cultured mammalian cells treated with eight organophosphorus pesticides. Mutation Research 88:307-316.

Test Chemical:

Di-Syston 98.6%.

Experimental Protocol:

Tester Cell Lines: The cell lines used in this study were identified as Burkitt lymphoma cell line B35M (2n = 47 to 49), normal human lymphoid cell line Jeff (2n = 46) and Chinese hamster cell line V79 (2n = 21 to 23). However, only the Chinese hamsters cell line V79 was used with the test material because a prior assay indicated that similar trends were exhibited for sister chromatid exchange (SCE) in all 3 lines.

Medium: The growth medium used was identified as RPMI 1640 supplemented with 10 percent fetal calf serum, 100 U/ml penicillin and 100 ug/ml of streptomycin.

SCE Assay:

Cell Growth and Treatment: To rapidly growing V79 cells in a series of 25 $cm^2$ tissue culture flasks, 10 ul/ml (final concentration) of 5-bromo-deoxyuridine (BudR) and the test material were added. Treatment of the cultures was for approximately two cell cycles (29 hours). Control cultures with BUdR only and BUdR plus DMSO were also included. Colcemide (0.04 ug/ml) was added to all cultures 2 hrs before harvesting for SCE analysis. Harvesting was after 2 rounds of replication in the dark after treatment with BUdR and the test material.

Slide Preparation and Evaluation: The technique used for slide preparation was previously described[1]. Coded slides stained to reveal chromatid exchange in the metaphase stage were examined under an oil immersion objective. SCEs at the centromere were not scored because of difficulty in making a distinction between exchanges and chromatid twisting.

Using staining patterns, SCE frequencies in BUdR only, BUdR plus DMSO and BUdR plus the test material were determined. Characteristics of first, second and third mitoses (required interpretation) were defined as follows: $M_1$ (first mitosis) - both chromatids were darkly stained; $M_2$ (second mitosis) - had differential staining of sister chromatids, i.e., one darkly and one lightly stained; and $M_{3+}$ (third and further mitoses) - only one-fourth or less of all chromatids were darkly stained. Analyses at each treatment level was performed on 100 randomly selected metaphases.

Preparation of Test Material: Disyston was solubilized in DMSO and delivered at a final doses of 10, 20, and 40 ug/ml in Experiment No. 1, and at doses of 10, 20, 40, and 80 ug/ml in experiment No. 2.

RESULTS:

The results in this study were reported as mean values for SCE/cell and the range at each level of treatment was given. There were two experiments performed with Disyston as the test material.

In experiment No. 1, there were no data reported on the number of cells in $M_1$, $M_2$, or $M_3$ phrases of mitosis. Fifty cells were analyzed at each dose and the mean SCE/cell values were not statistically different from the solvent control value which was 4.8 $\pm$ 0.3*.

In experiment No. 2 the cell percentages in $M_2$ of mitosis were 100 percent in the untreated control and 98 percent in the DMSO sample; in the treatment groups, between 94 percent (80 ug/ml) and 98 percent (10 ug/ml) were in $M_2$ of mitosis. The solvent control SCE/cell value was 5.1 $\pm$ 0.3 compared to 5.9 $\pm$ 0.4 (80 ug/ml Disyston) and 5.5 $\pm$ 0.3 [illegible] ug/ml Disyston).

There was no standard positive control chemical used, however, six of the 8 organophosphorus pesticides induced SCE at an increased level. Demeton, for example displayed a dose-responsive increase in SCE/cell as follows: 10 ug/ml (7.8 $\pm$ 0.4), 20 ug/ml (9.2 $\pm$ 0.4), and 40 ug/ml (13.2 $\pm$ 0.6), (19.7 $\pm$ 0.6). The negative control value was (4.2 $\pm$ 0.3). Similar responses were seen for Methylparathion, Trichlorfon, and Dimethoate. Malathion and methidathion induced SCE, but only at relative high doses.

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[1]Huang C.C. and Furukawa M. 1978. Exptl. Cell Res. 111:458-461.

*Mean value $\pm$ S.E.M.

DISCUSSION:

The authors concluded that Disyston® did not induce SCE in V79 hamster cells under the conditions of the study, but that it caused a delay in the cell cycle, i.e., at 80 ug/ml, 6 percent of the cells failed to progress from $M_1$ to $M_2$ in 29 hours. At the same dose, diazinon treatment caused 100 percent of the cells to remain in $M_1$.

Our assessment is that the author's conclusions were correct, assuming that the mean values reported for SCE/cell were substantiated by raw data. The positive-responding organophosphate pesticides served as evidence for the assay's sensitivity, making a standard positive control unnecessary for proving that the assay was working. In addition, it is noteworthy the Demeton is an oxidized metabolite of Diyston®. Hence, the positive induction of SCE by Demeton suggests that the test material might have induced significant SCE in the presence of a metabolic activation system.

CONCLUSIONS:

Under the conditions of the assay as reported in this study, Disyston® did not appear to induce SCE in Chinese hamster ovary cells at doses of 10, 20, 40, or 80 ug/ml. However, no assays were performed using rat liver S9 activation and a positive response by Demeton indicates that oxidation of Disyston would produce a genotoxic product.

CLASSIFICATION: Acceptable for treatment in a nonactivated system; however, unacceptable for metabolic activation.

# Rec-Assay in B. subtilis, and Reverse Mutation Assay in S. typhimurium

Fiche Master ID 000000000

Inukai, H.; Iyatom, A. (1976). Disulfoton mutagenicity test on bacterial systems. An unpublished report (No. AC 86190) prepared by Nitokuno Agricultural Chemicals Institute, Toyoda, Japan, prepared for Mobay Chemical Corp., Stilwell, KS. Dated June 30, 1976.

Test Chemical:

Disulfoton technical 94.1%.

Experimental Protocol:

Rec-Assay:

Bacterial Strains: The assay used two strains of Bacillus subtilis, NIG 17, and NIG 45. NIG 17 is a recombination repair-deficient strain ($Rec^-$); NIG 45 is the wild type.

Preparation of Test Material: The test material was dissolved in dimethylsulfoxide (DMSO), and the stock solution concentrations were unspecified.

Controls: Mitomycin C (MC) was dissolved in distilled water and used as a positive control. No negative (solvent) control was used.

Assay: The assay was conducted using the procedures of Kada et al[1]. Overnight cultures of the strains were streaked on solid agar plates (composition not specified), and a paper disc saturated with the appropriate amount of the test material (3, 30, or 300 ug/disc) and placed on the edges of the smears. The plates were incubated overnight at 37°C and growth inhibition from the centered was disc measured in mm. A positive control 0.3 ug MC/disc of was included.

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1Kada, T. et al. 1972. Mut. Res. 16:165-174.

Reverse Mutation Assay:

Bacterial Strains: The assay used Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100.

Preparation of S9: The S9 fraction was prepared from the liver of unspecified strains of rats and mice which had been treated with an unspecified dose of phenobarbital. This fraction was combined with glucose-6-phosphate, NADP, and phosphate buffer to prepare the S9 mix.

Preparation of the Test Material: The test material was dissolved in DMSO and the stock solutions were unspecified.

Controls: Although it was not specified, apparently DMSO was used as a negative (solvent) control. The following compound specified final concentrations were used as positive controls.

Table 1. Positive Control Chemicals

| Chemical | Concentration ug/plate | Strain Tested |
|---|---|---|
| Furylfuramide (AF-2) | 0.02 | TA 100[a] |
| Dexon | 50 | TA 1537[a], TA [illegible][a] |
| N-methyl-N'-nitro-N-nitrosoguanidine (NTG) | 10 | TA 1535[a] |
| Dimethylnitrosoamine (DMNA) | 1000 | TA 1535[c], TA [illegible][c] |
| "AAF" | 50 | TA 98[c] |

[a]Without metabolic activation.
[b]With and without metabolic activation using mouse S9.
[c]With and without metabolic activation using rat S9.

Nonactivated Assay: It was stated that the assay was performed according to the procedure of Ames et al.[2] However, only a single dose of disulfoton was tested (1000 ug/plate). No other details were provided.

[2]Ames et al., 1973a. Proc. Natl. Acad. Sci. USA 70:782-786; Ames et al. 1973b. Proc. Natl. Acad. Sci. USA 70:2281-2285; Ames et al. 1975. Proc. Natl. Acad. Sci. USA 72:979-983.

Activated Assay: For the activated assay, 0.1 ml of overnight culture of each strain, 0.3 ml of the S9 mix (rat or mouse), and 0.1 ml of the appropriate concentration of test material (0.1, 10, 1000 ug/plate) or positive control chemical were spread on a plate of solid agar. The plates were incubated at 37°C for 48 hours and the revertant colonies counted.

Evaluation Criteria: No criteria for the evaluation of the results were given.

RESULTS:

Rec-assay: No inhibition of growth was seen in the plates containing disulfoton at 3, 30, and 300 ug/disc. The growth inhibition by 0.3 ug/disc of MC was 1 and 10 mm in strains NIG 17 and NIG 45, respectively (a difference of 9 mm).

Reverse Mutation Assay: The following results were obtained for the assay using rat S-9 (apparently duplicate were not used):

Table 2. Results of Reverse Mutation Assay - Rat S9 Used for Metabolic Activation

| Chemical | Concentration (ug/plate) | S9 | Revertants Per Plate TA 1535 | TA1537 | TA 98 | TA 100 |
|---|---|---|---|---|---|---|
| DMSO | -- | - | 15 | 18 | 35 | 411 |
| | -- | + | 13 | 24 | 26 | 476 |
| Disulfoton | 0.1 | + | 21 | 11 | 55 | 482 |
| | 10 | + | 17 | 12 | 48 | 325 |
| | 1000 | + | 22 | 13 | 22 | 395 |
| AF-2 | 0.02 | - | -- | -- | -- | >1000 |
| NTG | 10 | - | >1000 | -- | -- | -- |
| Dexon | 50 | - | -- | 501 | >1000 | -- |
| AAF | 50 | + | -- | -- | >1000 | -- |
| | 50 | - | -- | -- | 43 | -- |

The following results were obtained for the assay using mouse S9:

Table 3. Results of Reverse Mutation Assay - Mouse S9 Used for Metabolic Activation

| Chemical | Concentration (ug/plate) | S9 | Revertants Per Plate TA 1535 | TA1537 | TA 98 | TA 100 |
|---|---|---|---|---|---|---|
| DMSO | -- | - | 2 | 5 | 26 | 222 |
| | | + | 6 | 5 | 25 | 162 |
| Disulfoton | 0.1 | + | 6 | 5 | 25 | 224 |
| | 10 | + | 6 | 9 | 22 | 238 |
| | 1000 | + | 8 | 5 | 34 | 238 |
| AF-2 | 0.02 | - | -- | -- | -- | >1000 |
| NTG | 10 | - | >1000 | -- | -- | -- |
| Dexon | 50 | - | -- | 483 | >1000 | -- |
| AAF | 1000 | + | >1000 | -- | -- | >1000 |
| | 1000 | - | 10 | -- | -- | 223 |

DISCUSSION:

The authors concluded that under the conditions of the assay, disulfoton did not induce DNA-damage at concentrations of 3 to 300 ug/disc in the B. subtilis Rec-assay. They also concluded that Disulfoton did not induce reverse mutations in the S. typhimurium system of Ames at concentrations from 0.1 to 1000 ug/plate in the presence of rat or mouse liver S9 or at 1000 ug/ml in the nonactivated system.

Our assessment is that the authors have correctly interpreted their limited data, i.e., DNA damaging and mutagenic effects were not induced by the test material under the conditions of the assay. However, the DNA-damaging assay used a single plate at each concentration and should at least be in duplicate. The reverse mutation assay was only done with single plates at each concentration although activation was with mouse and rat S9. The preferred treatment of rodents is with Aroclor 1254 prior to S9 preparation; however, phenobarbitol was used instead. In our opinion, the reason for using this inducer should have been justified. In addition, strain TA 100 had spontaneous revertant levels that greatly exceeded the historical values seen in most

laboratories, e.g., about 120 to 160 colonies should be seen in solvent control plates and not more than about 180 in the presence of S9. As seen in Table 2, solvent control values were 411 and 476 in nonactivated and activated assays, respectively. The values for TA 100 of 222 and 152 for nonactivated and activated solvent controls (Table 3) are minimally acceptable. Perhaps triplicate or even duplicate plates would have resulted in more acceptable values. All other tester bacteria had suitable solvent control revertant values and the positive control value indicated that the assays could detect a positive response. A preliminary cytotoxicity assay and solubility determination should also have been included to justify the assay dosages.

CONCLUSIONS:

In the rec-assay with B. subtilis, no inhibition of growth was found at 3, 30, or 300 ug/disc disulfoton (the active ingredient of Disyston). The response of the assay to a positive control substance indicated the ability of the system to respond to a known mutagen. Therefore a DNA-damaging effect did not occur under the conditions of the assay. In the reverse mutation assay, the results suggested a negative response. However, replicate plates at the various dose levels were not used and the dose range chosen may have been inadequate.

CLASSIFICATION: Unacceptable.

The rec-assay had no solvent control and was not done in replicate plates; the reverse mutations assays were by single plates and both dosage and activation appeared to be inadequate.

003958

Dominant Lethal Test for Male Mouse

Fiche Master ID 000000000

Herbold, B. (1980). Dominant lethal test on male mouse to evaluate S 276 for mutagenic potential. Report No. 9440 prepared by Bayer AG Institute fur Toxicology for Mobay Chemical Corporation. Dated September 23, 1980.

Test Chemical:

Di-Syston technical 94.9%.

Experimental Protocol:

Preparation of the Test Material: The test material, Disyston® was prepared for oral administration in a 0.5 percent Cremophor emulsion so that the dosed group received 5 mg S 276/kg in a volume of 10 ml/kg.

Animal Speci- and Maintenance: The mice were of strain NMRI/ ORIG Kissle- -nd were supplied by S. Ivanovas GmbH, Kisslegg/Allgau. The males weighed 33 to 40 g and the females weighed 20 to 33 g and were 8 to 12 weeks old at the start of the study. Each group contained 50 males; the negative control group had 600 females and the S 276-treated group had 595 females.

Makrolon cages (Type I) marked for identification were used to house each male with one virgin female and post-mating, the females were caged singly; the temperature was maintained at 23 ± 1°C with a relative humidity of 44 to 54 percent and a 12 hour dark/light cycle.

Preparation of the Test Material and Dosing: The test material, S 276, was formulated in 0.5 percent Cremophor emulsion. Dosing of the males was by a single oral dose at 5 mg S 276 per kg and control males received only the vehicle; both the test substance and vehicle control were administered in a volume of 10 ml/kg. The dose of S 276 was selected based on the results of a range-finding study with male mice using 5 mg/kg and 3 mg/kg, respectively; only mild symptoms were observed at the higher dosage. Females were all untreated.

Mating Procedures and Implantation Assessments: Males were assigned to the control and test groups using a randomization plan designed and implemented by the PH Documentation and Biometrics Department. Beginning at the first day of dosing with S 276, 12 matings of 4 day duration with different females were performed; the duration of this phase was for 48 days. This procedure theoretically allowed testing of all stages of the germ cells in male. Females were not examined for the presence of vaginal plugs.

Females were examined at about 14 days from the midpoint of the mating period to determine pre- and post-implantation losses (assessment criteria). From the sums of deciduomata, resorptions and dead embryos the number of dead implants was calculated. Scoring including corpora lutea, viable implants, dead implants, and total implants.

Biometrics: For the dead and total implants (transformed by square root) and the ratio of dead to total implants (angular transformation), and analyses were performed by a 2-factor analysis of variance. When analysis variance was significant at $p < 0.05$ for differences between groups, the Dunnett test was used to determine least significance difference. When the F-test showed a significant difference at $p < 0.05$, the Tukey test was used. Since a second test material (in addition to S 276) was assayed in this study, a common control was analyzed versus both test substances by analysis of variance. Frequency distributions of different parameters were also compared by the non-parametric Kolomogorov-Smirnov test.

RESULTS:

General Tolerance Effects: Dosing of 5 mg/kg with S 276 had no visible effects on motor activity and there were no compound-related mortalities, "only mid poisoning symptoms of brief duration, with mild drowsiness being observed for up to 1 hour post-treatment." One female from the period 7 mating had a cherry-sized ovarian tumor (females were untreated) and was not considered in the assessment. In addition, there were some female mice that received bites from their male mating partner and two of the males had ruffled coat hair.

Fertilization Index: This parameter was defined as follows:

$$\frac{\text{Number of fertilized females}}{\text{Number of females tested}} \times 100$$

The fertilization index reported for females mated with males that received 5 mg S 276/per kg was 437/692 (73.8 percent) compared with 442/600 (73.7 percent) in the vehicle control group. There was no compound related effect on the fecundity of males.

Pre-implantation Losses: The methods or estimation of this parameter was by: (a) corpora minus implantation or (b) comparisom of control and treated implantations. Method 'b' was considered by the authors to be the preferred one, but both methods were used so that method 'a' provided an additional check.

The corpora lutea in the control vs dosed animals were 10.9 versus 11.0 per fertilized female, respectively. The average number of implantations per female were 10.5 and 10.7 in the control and the dosed groups, respectively. The calculated pre-implantation losses were 0.36 and 0.31 in the control and dosed groups, respectively.

Post Implantation Losses: This was considered to be the most important criterion for assessing a dominant lethal mutagenic effect in the mouse model system. For this parameter, the sum of the deciduomata, resorption, dead embryos, and dead fetuses were considered.

From all twelve mating periods, the average number of living implants per fertilized female was 9.9 in the negative control group versus 10.0 in the group mated with dosed males (5 mg S 276/kg). In all twelve mating periods, the average number of dead implants per fertilized female was 0.60 in the negative control group versus 0.68 in the group mated with treated males (5 mg S 276/kg). Among all implants in the control group, there were 276/4660 or 5.7 percent dead implants versus 299/4681 or 6.4 percent dead implants in the S 276 treatment group.

DISCUSSION:

The author's assessment of the results was that "S 276 administered to male mice in a single acute oral dose of 5 mg/kg body weight did not have any lasting adverse effect on the general behavioral patterns of the mice." No effects were observed on fertility nor on the number of dead implants, viable implants, preimplantation loss, or total implants. The report indicated that statistical analyses of the data revealed no differences between the negative control and S 276-treated group at the $p < 0.05$ level by analysis of variance or by F-test and concluded that the test material did not induce mutagenesis in the dominant lethal test in the male mice dosed orally at 5 mg/kg.

Our assessment is that the author had made an accurate interpretation of his data. The sampling period of twelve weeks was adequate to detect an effect; however, the dose used did not appear to be high enough because it was not a maximum tolerated dose (MTD). The author had determined that there were no compound-related mortalities and that a dosage of 5 mg S 276/kg did not produce any overt adverse effects. We consider that an MTD based on

the test data reported should be required when the range finding test does not give enough data to determine an MTD. In addition, no positive control, substance was concurrently tested in the study. Consequently, the assay's sensitivity cannot be evaluated.

CONCLUSIONS:

Under the conditions of the assay, a dosage of 5 mg S 276/kg did not appear to induce a mutagenic response in male mice. However, it is not evident that the dose level was high enough nor that the assay had an appropriate sensitivity to detect a positive mutagenic response.

CLASSIFICATION: Acceptable.

A positive control material was not included in the study and the single dose level used of the test material was not justified as adequate by prior toxicity testing data nor by the findings of the assay.

003958

Effect on Growth of Mammalian Cells

Fiche Master ID 00000C00

Huang, C.C. (1973). Effect on growth but not on chromosomes of the mammalian cells after treatment with three organophosphorus insecticides. Proc. Soc. Exp. Biol. Med. 142:36-40, 1973. A published study (No. AC 86185) submitted by Mobay Chemical Corp., Stilwell, KS.

Test Chemical:

Di-Syston 96.8%.

Experimental Protocol:

Cell Lines: Human hematopoietic cell lines (B411-4, RPMI-1788, and RPMI-7191) obtained from Roswell Park Memorial Institute were used in this study. The cells were derived from normal male individuals, and had normal diploid or pseudodiploid karotypes.

Preparation of the Test Materials: The test material was dissolved in dimethylsulfoxide (DMSO) at concentrations of 10 or 100 ug/ml just prior to use.

Controls: DMSO was used at a maximum concentration of 0.5 percent as a negative (solvent) control; methylmethanesulfonate (MMS) at a concentration of 10-80 ug/ml served as a positive control. One culture per assay received no treatment and served as an untreated control.

Assay: The test material was added to cultures in "rapid growth" to given final concentrations of 25, 50, 75, and 100 ug/ml. The additions were made initially, and then at approximately 6, 12, 24, and 48 hours later, an unspecified aliquot of cells was withdrawn from each cultures for the determination of cell viability by the trypan blue exclusion method and for chromosome study. The cultures were then washed and re-fed with test material-free medium; aliquots were then taken at 70, 95, and 140 hours after initial treatment. Two hours prior to the harvest of cells for chromosome study, the cultures were treated with 0.04 ug/ml colcemid. After sampling, the cells were swelled with sodium citrate, fixed with acetic acid-methanol, and stained with geimsa. Approximately 100 metaphase figures were examined from each culture for "gaps, breaks, exchanges, dicentrics, pulverization, etc."

Evaluation Criteria: No criteria for the evaluation of the results were specified.

RESULTS:

In cell lines B411-1, treatment with the test material reduced cell viability overtime in a dose-dependent manner when compared to either the vehicle or untreated controls which had similar viability. At 48 hours, the cultures treated with 25 or 50 ug/ml Disyston® had viabilities that were 50 and 15 percent of controls, respectively. Cultures treated with 75 and 100 ug/ml showed no viable cells at this time. After washing and refeeding, the culture treated with 25 ug/ml of test material resumed normal growth; the culture dosed at 50 ul/ml showed no growth. Although the investigator stated similar results were obtained with the other cell lines, none of these data were reported. In the chromosome aberration study, the following results were obtained for the control groups of cell lines B411-1:

Table 1. Chromosomal Aberrations in Control Cultures (Cell Line B411-4)

| Treatment | Time (hrs) | Concentration (ug/ml) | Percentage of Cells with: Gaps or Break | Pulverization |
|---|---|---|---|---|
| None | 6-48 | -- | 1-5 | 0-1 |
| DMSO | 6-48 | <0.5 percent | 1-5 | 0-1 |
| MMS | 6 | 10 | 3 | 1.0 |
| | | 20 | 4 | 2.3 |
| | | 40 | 18 | 14.5 |
| | | 80 | No mitosis | -- |
| | 12 | 10 | 8 | 1.4 |
| | | 20 | 11 | 31.2 |
| | | 40 | 25 | 74.0 |
| | 24 | 10 | 8 | 2.0 |
| | | 20 | 18 | 3.1 |
| | | 40 | 35 | -- |
| | 48 | 10 | 15 | 75.8 |
| | | 20 | No mitosis | -- |
| | | 40 | No mitosis | -- |

For the cell cultures treated with the test material, the investigator stated that no concentration produced an increase in chromosomal aberrations when compared to controls. No individual data were reported.

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DISCUSSION:

The authors stated that Disyston inhibited culture growth in B411-4 cells, but that normal growth ensued after removal of the test material. The data presented in this report illustrated this response at the various doses of the test material. The chromosomal aberrations data was, however, not presented. In addition, the investigator did not specify the criteria used to evaluate the data and for determining a positive response, although the data for MMS provided evidence for the assay's sensitivity.

Our assessment is that sufficient data on cytotoxicity was present, but that the author's claim of no effect on chromosomal or chromatid aberrations was not substantiated.

CONCLUSIONS:

In this study, data showed that Disyston® was cytotoxic to human hematopoietic cell line B411-4 at concentrations of 25-100 ug/ml. This claim was also made for 2 other cell lines although no data were given. Although results obtained with a positive control substance, methylmethanesulfonate, demonstrated the ability of the test system to detect chromosomal damage, there were no data on chromosomal or chromatid aberrations induced by Disyston®. The only useful information was that Disyston concentrations of 50 ug/ml or greater was cytotoxic for B411-4.

CLASSIFICATION: Unacceptable as negative evidence for chromosomal aberrations because no quantitative data was present in the report.

003958

Gene Mutation in Yeast

Fiche Master ID 000000000

Jagannath, D.R. (1961). Mutagenicity evaluation of S 276 (batch 25.10.1979, study T4003065) in the Saccharomyces cerevisiae reverse mutation induction assay. An unpublished report (No. 2087) prepared by Litton Bionetics, Inc., Kensington, MD, for Mobay Chemical Corp., Stilwell, KS. Dated October 1981.

Test Chemical:

Disulfcton, batch no 25.10.1979; provided by Bayer AG; no purity was given.

Experimental Protocol:

Yeast Strain: The assay was conducted using the methionine auxotrophs of Saccharomyces cerevisiae, strains S138 (frameshift mutant) and S211 that were derived from the prototrophic haploid strain S288c from R.K. Mortimer (University of California). Stocks of the strains were maintained as isolates at 4°C; working stock suspensions were obtained from stationary phase cultures grown at 30°C in yeast extract peptone. The selective medium was yeast minimal medium without methionine.

Preparation of the S9 Fraction: The S9 fraction was prepared from the livers of male adult Sprague-Dawley rats induced by Aroclor 1254 according to the procedure of Ames et al.[1] The S9 mix contained (per ml): NADP (4 umoles), glucose-6-phosphate (5 umoles), $MgCl_2$ (8 umoles), KCl (33 umoles), phosphate buffer (100 umoles), and 100 ul of S9 fraction.

Preparation of the Test Material: Preparation of the test material was not described.

Controls: The negatice (solvent) control was not specified. Positive control substances are shown in Table 1.

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[1](1975. Mut. Res. 31:347-364).

TABLE 1. Positive Control Substances

| Assay | Chemical | Amount per Well | Strain |
|---|---|---|---|
| Nonactivated | Quinacrine mustard (QM) | 50 ug | S138 |
| | Ethylmethane sulfonate (EMS) | 10 ug | S211 |
| Activated | Cyclophosphamide (CP) | 50 ug | S211 |
| | 2-Acetylaminofluorene (2-AAF) | 10 ug | S138 |

Reverse Mutation Assay:

Nonactivated: Doses were selected based on a preliminary cytotoxicity test in which the number of survivors was determined on complete medium afte a 60-minute exposure to the test material. No cytotoxicity was observed at concentrations up to 150 ul/plate. The dose range tested was given as 1.5 to 300 ul/well; precisely which doses were tested was not specified. An aliquot of the appropriate test material concentration (50 ul), $2 - 5 \times 10^8$ yeast cells of either strain, and 0.6 - 1.0 ml of phosphate buffer were placed in each of 7 wells in a Costar dilution well plate (wells 16 mm in diameter, and 13 mm deep). The total volume per well was 1.5 ml. Two wells contained the positive or negative control substances. The plate was incubated with shaking for 60 minutes at 37°C, after which the plate was cooled on ice and 1.0 ml of the contents of each well as removed and spread on 4 plates of yeast minimal selective medium. The plates were incubated at 30°C for 4-6 days; after incubation, the number of revertant colonies were counted. Toxicity was determined by diluting the cell suspension of the well contents on yeast complete medium and counting the colonies resulting after incubation at 30°C.

Activated: This assay was conducted in the same way as the nonactivated assay with the substitution of 0.6 - 1.0 ml of the S9 mix for phosphate buffer.

Evaluation Criteria: The test material was considered mutagenic if positive responses, i.e., increases in the number of revertant colonies per ml of cell suspension plated compared to the negative control, were observed at 3 concentrations and if the highest increase was equa' to twice the solvent control value. In addition, positive results had to be reproducible.

RESULTS:

In the nonactivated assay, the following results were obtained (because footnotes were omitted from the report, data on the amount of test material per plate were not available).

| Chemical | Concentration | Strain S138 | | S211 | |
|---|---|---|---|---|---|
| | | Survivors[a] | Mutant[a] | Survivors | Mutant |
| Control | -- | 466 | 7 | 176 | 58 |
| Disulfoton | ( ) | 316-510 | 4-19 | 164-263 | 54-91 |
| QM | 50 ug/plate | 35 | 519 | --[b] | -- |
| EMS | 10 ug/plate | --[b] | -- | 183 | 1720 |

[a]Total count for 4 plates.
[b]Not tested in this strain.

In the activated assay, the results were as follows:

| Chemical | Concentration | Strain S138 | | S211 | |
|---|---|---|---|---|---|
| | | Survivors[a] | Mutant[a] | Survivors | Mutant |
| Control | -- | 481 | 19 | 228 | 71 |
| Disulfoton | ( ) | 432-672 | 9-17 | 193-310 | 73-80 |
| QM | 50 ug/plate | --[b] | -- | 237 | 127 |
| EMS | 10 ug/plate | 694 | 12 | --[b] | -- |

[a]Total count for 4 plates.
[b]Not tested in this strain.

DISCUSSION:

The results of this study suggest that the test material did not produce a mutagenic effect under the conditions of the assay; however, data on the amount of test material per test plate were not available, although it was stated that the amounts ranged from 1.5 to 300 ul/plate. In addition, the solvent control was not identified and the activation test results using CP or 2-AAF did not appear to induce a mutagenic response. Hence, the sensitivity of the assay in the presence of S9 may have been inadequate.

CONCLUSION:

The results of the assay, a reverse mutation test in S. cerevisiae strains S138 and S211, suggested that disulfoton, the active ingredient of Disyston, was not mutageic at stated doses of 1.5-300 ul/plate. However, the sensitivity of the assays in the presence of S9 activation did not appear to be adequate based on lack of response by positive control chemicals. In addition, critical information on the amount of test compound used per test plate was not reported in association with the results of each test.

CLASSIFICATION: Unacceptable.



[redacted]

Mutagenicity (Non-Disjunction)

003958

Fiche Master ID 000000000

Brusick, D.J. (1961). Mutagenicity evaluation of S 276 Batch 25.10. 1979 in the mitotic non-disjunction in Saccharomyces cerevisiae strain D6. Revised final report on Study No. T3003064 prepared by Litton Bionetics, Inc. for Bayer AG Institute fur Toxicology. Dated October, 1981.

Test Chemical:

The test material was identified as S 276 Batch 25.10. 1979 Study No. T3003064, and was described as a clear yellow liquid, received on May 11, 1981.

Experimental Protocol:

Test Strain: Saccharomyces cerevisiae strain D6, a diploid yeast strain was used in the assay. Its genotype is:

Chromosome III his 4: a
=====  =

Chromosome VII ada 3: leu 1 trp 5 cyh 2 met 13
=====  ==========
+  +  +  +  +

Chromosome XV ade 2-40
========
ade 2-40

The loss of chromosome VII is the event interpreted to be non-disjunction. When plate on an appropriate growth medium this strain produces red colonies that are sensitive to cycloheximide. If chromosome VII is lost, white colonies arise that are resistant to cyclohexamide. Loss of both arms can be verified by plating onto a minimal medium lacking leucine, tryptophan, or methionine.

Media: The standard yeast growth medium used was that which was described by Zimmerman[1]. The development of white cycloheximide resistant colonies was assessed on complete medium fortified with 2 ug/ml cycloheximide. The amino acid requirements, linked to the cycloheximide gene, were asssessed on minimal medium[2].

Metabolic Activation: Rat liver S9-Mix, as described by Ames et al.[3], was used.

Preparation of Test Material: Preparation of the test material was not described in this revised final report. However, the authors stated that five doses that caused no more than 50 percent cytotoxicity were used.

Non-disjunction Assay: Stationary phase yeast cells, harvested after 4 days growth at 30°C on complete nutrient agar were harvested, washed twice in distilled water, and resuspended in growth medium. In the final treatment tubes, there were approximately $10^7$/cells/ml in growth medium.

In the nonactivated system there was 0.5 ml of phosphate buffer at pH 7.4 in the mixture of cells and test material. In the activation assay 0.5 ml of S9-Mix was substituted for buffer. The tester yeast were treated with the appropriate concentration of test material, either with ot without S9, for 3 hr at 37°C, then plated on complete medium and complete medium plus cycloheximide. The plates were incubated for 4 to 5 days at 30°C and then the colonies were scored for viability and cycloheximide resistance white colonies or white sectors). The procedure included concurrent positive and solvent (negative) controls.

Data Evaluation: The test was considered to be positive if an aneuploid frequency of 1.5 times or greater than the solvent control was induced by the test compound.

By assessing the red cycloheximide-resistant colonies and the white cycloheximide colonies that were not deficient for all three nutritional markers, mitotic recombination could also be quantified and evaluated.

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[1]Zimmerman, F.K. 1973 in Hollander, A. ed. Chemical Mutagens: Principles and Methods for their Detection. Vol. 3, Plenum Press, NY, pp. 209-258.

[2]Parry, J.M. and Zimmerman, F.K. 1976. Mutation Res. 36:49-66.

[3]Ames, B.N., McCann, J., and Yamasaki, E. 1975. Mutation Res. 35:347-364.

RESULTS:

The authors stated that the results were presented in Tables 1, 2, 3, and 4. However, only Table 1, entitled "Toxicity Evaluation" and dated 5-21-81 was presented for the test material, S 276 Batch 25.10.1979 Study No. 730003064. There were 14 doses of the test material which were serially diluted by 1:2 which ranged from 0.018 to 150 ul/tube and a negative control. Since the survival indices were between 0.8 and 1.6 (based on a control index of 1.0 indicating no toxicity) no dose tested within this range could be considered to have greater cytotoxicity than was set in the minimum requirement for a valid assay.

DISCUSSION:

This report did not include data on the induction of aneuploidy or induction of mitotic recombination. Hence, it could not be evaluated. There was a section in which the author evaluated the results presumed to be present in the final report submitted in September, 1981. This revised report only contained Table 1 which was the concurrent cytotoxicity control.

CONCLUSIONS:

This report did not contain the data necessary for an evaluation.

CLASSIFICATION:

Unacceptable because there were no data involving mitotic non-disjunction presented.

# Metabolism of Di-Syston in the Mice

Fiche/Master ID 00083251

March, R.B.; Fukuto, T.R.; Metcalf, R.L. (1957) Metabolism of P-32-Dithio-systox in the White Mouse and American Cockroach: Submitter #1830. (Unpublished study received on unknown date under PP0244; prepared by Univ. of California - Riverside, Citrus Experiment Station, submitted by Chemagro Corp., Kansas City, Mo.; CDL: 098725-F)

Test Chemical:

$p^{32}$ radiolabeled Dithio-Systox (Di-Syston)

Experimental Protocol:

The metabolism of Di-Syston was studied *in vivo* and *in vitro* in the white mice. The chemical was administered by *intraperitoneal injection* of propylene-glycol solutions. The animals were caged singly in metabolism cages. Urine and feces were collected. The volume of the urine and weight of the feces were determined and the total radioactivity was measured. An aliquot of the urine was extracted with an equal volume of chloroform, the phases were separated, and evaporated for radioassay or concentrated for paper chromatography.

For the *in vitro* study, mouse liver slices were washed in the cold buffer, and cut into thin slices. According to the author, slices from one half of a mouse liver were transferred directly to a Warburg flask containing 2.9 ml Krebs-Ringer phosphate buffer, $10^{-2}$ M glucose, 0.1 ml of 20% (w/v) KOH in the center well, and 0.1 ml of $10^{-2}$ M alcohol solution of Di-Syston. The flasks were oxygenated for 5 minutes and then closed, and the metabolism was allowed to proceed for 2 to 16. The contents of the flask were homogenized and extracted with the equal volumes of chloroform, the phases were separated and the concentrated extracts were analyzed using paper chromatography.

Anticholinesterase potency of the resulting metabolites was determined as follows: after radiometric location of the separated compounds on the chromatogram, the areas containing ratioactivity were cut and diced directly into Warburg flasks containing the necessary ingredients for the determination of inhibition of house-fly head cholinesterase. Inhibitory activities were established by comparison with control runs and the concentrations of inhibitors were determined ratiometrically on aliquots of the fly-head homogenates.

Results:

The author reported summary data indicating that the urine metabolites consisted mainly of hydrolysis products which were inactive as cholinesterase inhibitors. A very small amount of the administered ratioactivity was recovered in feces.

In vitro metabolism data indicated the presence of Dithio-Systox sulfoxide, and sulfone, and the thiol analog sulfoxide and sulfone. The Dithio-Systox sulfoxide was present in greatest quantity followed by thiol analogue sulfoxide, Dithio-Systox sulfon, and thiol analogue sulfone.

The authors postulated the following metabolic pathway for Dithio-Systox in the mice based on the structure of Dithio-Systox and previous studies on isomers of Systox (March et al. 1955). It was possible to postulate certain biochemical transformations according to the following schematic diagram.

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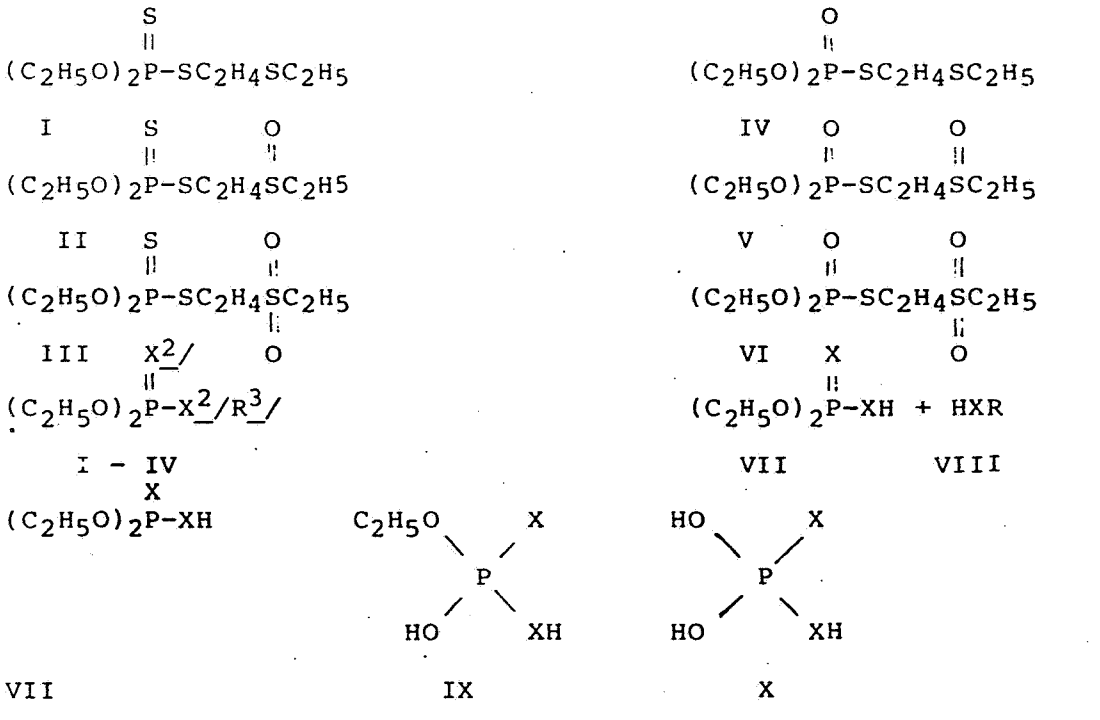


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2/ X = O or S

3/ R = $C_2H_4SC_2H_5$, $C_2H_4SOC_2H_5$, or $C_2H_4SO_2C_2H_5$

The transformation of Dithio-Systox (I) to its thiol analogue (IV), 0,0-diethyl S-2-(ethylthio)ethyl phophorothiolate (Systox thiol isomer), by oxidation of P = S to P = O, increases anticholinesterase activity more than a hundred fold (see Table 1).

The anticholinesterase activity of the metabolites was determined for the metabolites isolated from the in vitro reacting mixture. The $I_5$ values in molar concentration for the oxidative metabolites were determined using house-fly head cholinesterase. These values are presented in Table 1.

Table 1. Inhibition of house-fly head cholinesterase and average $R_{mc}$ a/ values by various paper chromatographic methods for Dithio-Systox and its metabolites.

| | | $I_{50}$ b/ molar Conc. | Average $R_{mc}$ a/ Values | | | |
|---|---|---|---|---|---|---|
| | | | A c/ | | B d/ | C e/ |
| | | | Petroleum Ether:Toluene | | | |
| | | | 4:1 | 7:3 | | |
| I. | $(C_2H_5O)_2P(S)-SC_2H_4SC_2)_5$ | > $1 \times 10^{-4}$ | 0.98 | .95 | .03 | .97 |
| II. | $(C_2H_5O)_2P(S)-SC_2H_4SC_2H_5$ | $7 \times 10^{-5}$ | 0.16 | .32 | f/ | f/ |
| III. | $(C_2H_5O)_2P(S)-SC_2H_4SC_2H_5$ (O) | $3.5 \times 10^{-6}$ | 0.52 | .65 | f/ | f/ |
| IV. | $(C_2H_5O)_2P(O)-SC_2H_4SC_2H_5$ | $3.5 \times 10^{-6}$ | 0.70 | .83 | f/ | f/ |
| V. | $(C_2H_5O)_2P(O)-SC_2H_4SC_2H_5$ | $1.5 \times 10^{-6}$ | 0.0-0.04 | .05 | f/ | f/ |
| VI. | $(C_2H_5O)_2P(O)-SC_2H_4SC_2H_5$ (O) | $6 \times 10^{-7}$ | 0.03-0.06 | .12 | f/ | f/ |

a/ $R_{mc}$, ratio of distance of travel from origin for maximum concentration of compound to that of solvent front.

b/ House-fly head cholinesterase.

c/ A = 50% propylene glycol system.

d/ B = 5% silicone system.

e/ C = plain paper system.

f/ Not differentiated, but chromatograph at or near solvent front.

Discussion and Conclusions:

The data were very limited and presented as summary tables. Obviously, the authors derived their conclusions based on the analogy of chemical structure of Di-Syston and other organophosphorus pesticides.

The metabolism of Di-Syston in mice involves at least two types of biochemical reactions. The first is the sequential oxidation of the thioether sulfur and/or oxidative desulfuration resulting in oxidative metabolites with greater anticholinesterase properties. The second is hydrolytic cleavage of the ester, most probably producing phosphoric acid, thiophosphoric acid and dithiophosphoric acid and rendering the molecule totally inactive as a cholinesterase inhibitor.

Core Classification:

Supplementary data.

003958

# The Metabolism and Excretion of Di-Syston by Rats

Fiche Master ID 000000000

Puhl, R.J. and Fredrickson, D.R. (1975). The Metabolism and Excretion and Excretion of Di-Syston by Rats. (Unpublished report submitted by Mobay Chemical Corporation), Report #44261, prepared by Chemagro Agricultural Division - Mobay Chemical Corporation, dated May 6, 1975.

Test Chemical:

Di-Syston-o-ethyl-1-$^{14}C$ with specific activity of 2.92 mCil/mM and radiochemical purity of more than 99%.

Experimental Protocol:

Twelve male and twelve female albino Sprague-Dawley rats with an average weight of 235 gm were given a single dose of the radiolabeled Di-Syston. The dose rates were 1.2 and 0.2 mg/kg for males and females respectively. The material was dissolved in 50% ethanol, and the dosing volume was 0.5 ml per animal. A single oral dose was administered directly into the stomach using a feeding needle. The animals were allowed food and water ad libitum throughout the experiment. For the short-term tissue study, plastic cages equipped for separate collection of urine and feces, were used.

In a separate experiment designed to study the excretion for an extended period of time, two males and two females were dosed, after which each animal was housed in a separate glass cage.

Urine and feces were collected at 4, 8, 12, and 24 hours and then at subsequent 24 hours intervals until sacrifice. The weight of feces and the volume of urine, was recorded. An aliquot of urine was removed for radioassay after each collection period.

Ethanolamine was used to trap carbon dioxide.

In the shor term tissue study, twelve males and twelve females were utilized, two of each being sacrificed at 1, 3,6, 12, 24 nad 48 hours after dosing. Two animals of the same sex were kept in each plastic cage, therefore, each of the urine and feces samples were composite of two rats. Urine and feces, in this short-term study, were collected only at the time of sacrifice, with the exception of the 48-hour animals from which a 24-hour sample was also taken.

Following decapitation, blood and tissues were collected. Tissues taken for analysis included; liver, kidneys, heart, brain, muscle, fat and skin. Liver samples were hemogenized in water and partitioned with chloroform. Tissues were homogenized and prepared for radioactivity determination. Aqueous samples such as urine, plasma, and sulfuric acid were diluted with water and counted. Blood and solid samples (except brain and fats) were combusted and counted. Brain and fat were solubilized and counted. Ethanolamine from the carbon dioxide traps was dilute with the counting cocktail and counted Aliquots of chloroform extracts of tissues were counted.

Liver and kidneys water homogenates were extracted with chloroform. Water soluble metabolites were separated by thin layer chromatography using cilica fel or cellulose plates using the following solvent systems:

A. Acetone:t-butanol:ammonium hydroxide:water (5:4:1:1)
B. Acetonitrile:water:ammonium hydroxide (80:18:2)
C. t-Butanol:acetonitrile:ammonium hydroxide (5:4:1)

The metabolites were quantitated by scraping the plate in sections using an Analabs TLC Zonal Scraper. The zones were counted as described in the Radiometric Analysis Section.

Thin layer chromatography of organosoluble activity was performed on silica gel $F_{254}$. Identification and quantitation was carried out as described for the watersoluble metabolites. Ethyl acetate:benzene (2:1) was found to give the best over-all separation of DI-SYSTON and its 5 oxidation products although the separation of (Disulfoton sulfoxide) and (Disulfoton oxygen analog sulfone) fair. Improved separation of the latter two was obtained using isopropyl ether:acetone (1:1). All standards were visualized by placing the developed plate in an iodine chamber.

The following standards were used for co-chromotography.

PSS - DI-SYSTON
PSSO - DI-SYSTON Sulfoxide
$PSSO_2$ - DI-SYSTON Sulfone
POS - DI-SYSTON Oxygen Analog
POSO - DI-SYSTON Oxygen Analog Sulfoxide
$POSO_2$ - DI-SYSTON Oxygen Analog Sulfone
DEP - Diethylphosphate
DEPT - Diethylphosphorothioate
DEPDT - Diethylphosphorodihioate
MEP-DEP - Monoethylphosphate-Diethylphosphate

RESULTS:

Excretion of Disulfoton: Rats given a single dose of $^{14}C$. Disulfoton, excreted the radioactivity primarily in urine. Tables 1 and 2 show the excretion pattern in males and females respectively. In males, a total of 84.3%, 6.1, and 9.2% of the administered dose was excreted in the urine, feces and expired air respectively in 10 days period. In females, a total of 78.9, 7.8, and 9.2% of the administered dose was excreted in the urine feces and expired air respectively in 10 days following dosing. The rate of excretions was significantly slower for females. Males excreted 50% of the administered dose in urine in the firs t4-6 hours, whereas, females required 30 to 32 hours.

Identification of Urinary Metabolites: The urinary metabolites were separated by thin layer chromatography and compared to standards by co-chromatography. Greater than 90% of the activity in the urine of both males and females was attributed to diethylphosphate and dietylphosphorothioate. Analysis of urine samples from several sampling intervals indicates that as time progresses, the ratio of diethylphosphate to diethylphosphorothioate increases.

Chloroform extract of urine showed that a small chromatographic separation of the chloroform extract resulted in two major zones, one of which was attributed to Di-Syston oxygen analog sulfoxide, the second zone could not be unequivocally assigned but probably was due to either Di-Syston sulfoxide, Di-Syston oxygen analog sulfone, or a mixture of them. However, using a different solvent system, the activity could be separated into four zones that co-chromatographed with Di-Syston oxygen analog sulfoxid, Di-Syston sulfoxide; Di-Syston oxygen analog sulfon, and some activity remained at the origin.

Nature of Fecel Metabolites: The identity of fecal metabolites was not investigated since elimination of the pesticide in the feces is not considered a major route of elimination.

Tissue Residues: Results of the liver and kidney tissues are shown in table , and respectively. According to the authors, when water homogenates of the liver partitioned with chlorofom, only 50% or less of the total radiocarbon residues in liver was found to be in the chloroform and water phases in both males and females. The remaining portion was extractable. Most of the the extractable residue was found to be in the water phase, and relatively very little in the organic phase. About 70-80% of the total radiocarbon residues was extractable in water and chloroform, with most of the radioactivity remaining in the aqueous phase.

DISCUSSION:

The labeling site is not considered appropriate since the O-ethyl is likely to be lost via O-dealkylation. This is a very common reaction for organophosphorus pesticides.

The major metabolites of DI-SYSTON-O-ethyl-1-$^{14}C$ in male and female rats were diethylphosphate (DEP) and diethylphorothioate (DEPT). These products formed from hydrolysis of DI-SYSTON and/or its oxidation products, a well-documented detoxication mechanism for organophosphorus insecticides.

DEPT could be derived from direct hydrolysis of Di-Syston or, alternatively, Di-Syston could first be oxidized to the sulfoxide (PSSO) and/or the sulfone which then could be hydrolyzed. The data suggested that the latter was occurring since the organosoluble fractions of liver and urine contained no Di-Syston.

Since PSSO but no PSO (Di-Syston oxygen analog) was detected in organosoluble fractions from rats, it is likely that rats are capable of rapidly oxidizing Di-Syston to the sulfoxide.

Once the PSSo was formed, it could be hydrolyzed to DEPT or oxidized to $PSSO_2$ or POSO. The organosoluble activity from liver contained, at most, trace amounts of $PSSO_2$ (Di-Syston sulfon) while POSO (Di-Syston oxygen analog sulfoxide) was a major component. The fact that $PSSO_2$ was not present in significant amounts implied that it played a minor role in the overall metabolic scheme. It was possible, however, that its rate of decomposition by hydrolysis to DEPT or oxidation to $POSO_2$ was faster than its rate of formation precluding any measurable build-up.

Hydrolysis of POSO would produce DEP while oxidation would give $POSO_2$. The latter compound, which was the major component of the organosoluble liver activity, would then serve as a second hydrolytic source of DEP.

Another potential pathway to the formation of DEP that should be considered involved conversion of DEPT to DEP. This mechanism was unlikely, however, as other workers have found that administration of DEPT-$^{32}P$ to mice[13] or rats[14] resulted in recovery of >95% of unchanged DEPT in the urine.

There appeared to be no major sex-related difference in the metabolism of DI-SYSTON since th distribution of radiocarbon in excreta and expired gases was similar to males and females and the major difference in the rate of excretion, females eliminating the activity slower than males.

The elimination of 9.2% of the dose as $^{14}CO_2$ suggested that a parallel metabolic pathway involved the hydrolytic formation of ethanol which was then metabolized to $CO_2$.

CONCLUSIONS:

According to the author's "administration of a single oral dose of DI-SYSTON-O-ethyl-1-$^{14}C$ to rats resulted in recovery of 96-99% of the dose as excretion products; 81.6% in urine, 7.0% in feces and 9.2% as expired $CO_2$. Excretory pathways were similar for males and females but the rate of excretion was slower for females.

Diethylphosphate (DEP) and diethylphorothioate (DEPT) comprised 93% of the urinary activity while minor urinary metabolites included the oxygen analog sulfoxide (POSO), oxygen analog sulfone ($POSO_2$) and DI-SYSTON sulfoxide (PSSO). The ratio of DEP to DEPT in urine was higher for females than for males.

Tissue and blood levels peaked at about six hours after administration of the dose. Females accumulated a larger percent of the dose in the liver than males. Organosoluble metabolites in liver were identified as PSSO, POSO, and $POSO_2$.

The higher toxicity of DI-SYSTON to females ma, be attributed to their lower capability for hydrolytic detoxification of the organophosphorus compounds.

CORE CLASSIFICATION:

Supplementary data. All data were presented as summary table. The study was also of a limited scope.

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Parts of this review, tables and figures were taken from the original report.

TABLE I

Excretion of $^{14}C$ Following Administration of a Single Oral Dose of DI-SYSTON-O-Ethyl-1-$^{14}C$ (Male Rats)

| Recovery of Radiocarbon, % of Total Dose | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Urine | | | Feces | | | Expired Gas ($CO_2$) | | |
| Animal 1 | Animal 2 | Average | Animal 1 | Animal 2 | Average | Animal 1 | Animal 2 | Average |
| 43.6 | 39.5 | 41.6 | 0.1 | <0.1 | | | | |
| 20.0 | 18.6 | 19.3 | 1.9 | 1.2 | 1.6 | | | |
| 5.3 | 6.6 | 6.0 | 1.2 | 1.4 | 1.3 | | | |
| 5.9 | 7.9 | 6.9 | 1.3 | 1.9 | 1.6 | 5.7[1] | 8.1[1] | 6.9[1] |
| 5.5 | 5.6 | 5.6 | 0.9 | 0.9 | 0.9 | 1.0 | 0.7 | 0.8 |
| 2.4 | 2.1 | 2.2 | 0.4 | 0.4 | 0.4 | 0.6 | 0.7 | 0.6 |
| 0.9 | 1.2 | 1.0 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 |
| 0.5 | 0.6 | 0.6 | 0.1 | 0.1 | 0.1 | 0.3 | 0.3 | 0.3 |
| 0.5 | 0.4 | 0.4 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 |
| 0.3 | 0.3 | 0.3 | | | | 0.2 | 0.1 | 0.2 |
| 0.2 | 0.2 | 0.2 | | | | | | |
| 0.2 | 0.2 | 0.2 | | | | | | |
| 0.1 | | | | | | | | |
| | | | | | | <0.1 | <0.1 | |
| 85.4 | 83.2 | 84.3 | 6.1 | 6.1 | 6.1 | 8.1 | 10.2 | 9.2 |

very: Urine + Feces + $CO_2$ - Animal 1, 99.6%; Animal 2, 99.5%; average, 99.6%.

ıanolamine sample was taken at 24 hours.

TABLE II

Excretion of $^{14}C$ Following Administration of A Single Oral Dose of DI-SYSTON-O-Ethyl-1-$^{14}C$ (Female Rats)

| Hours Post-Treatment | Recovery of Radiocarbon, % of Total Dose | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Urine | | | Feces | | | Expired Gas ($CO_2$) | | |
| | Animal 1 | Animal 2 | Average | Animal 1 | Animal 2 | Average | Animal 1 | Animal 2 | Average |
| 0 - 4 | 9.6 | 2.8 | 6.2 | N[1] | <0.1 | | | | |
| 4 - 8 | 8.2 | 1.8 | 5.0 | N | N | | | | |
| 8 - 12 | 10.6 | 14.3 | 12.4 | N | N | | | | |
| 12 - 24 | 19.9 | 19.9 | 19.9 | 2.7 | 2.4 | 2.6 | 3.2[2] | 3.3[2] | 3.2[2] |
| 24 - 48 | 18.6 | 16.9 | 17.8 | 2.2 | 2.9 | 2.5 | 2.0 | 1.2 | 1.6 |
| 48 - 72 | 5.5 | 8.6 | 7.0 | 1.5 | 1.3 | 1.4 | 1.0 | 1.3 | 1.2 |
| 72 - 96 | 3.2 | 5.1 | 4.2 | 0.5 | 0.6 | 0.6 | 0.6 | 0.9 | 0.8 |
| 96 - 120 | 1.8 | 2.0 | 1.9 | 0.2 | 0.6 | 0.4 | 0.8 | 0.9 | 0.8 |
| 120 - 144 | 1.3 | 1.8 | 1.6 | 0.2 | 0.1 | 0.2 | 0.2 | 0.4 | 0.3 |
| 144 - 168 | 1.0 | 1.3 | 1.2 | 0.2 | 0.3 | 0.2 | 0.7 | 0.5 | 0.6 |
| 168 - 192 | 0.7 | 0.7 | 0.7 | | | | | | |
| 192 - 216 | 0.6 | 0.6 | 0.6 | | | | | | |
| 216 - 240 | 0.4 | 0.5 | 0.4 | | | | 0.6 | 0.8 | 0.7 |
| Totals | 81.4 | 76.3 | 78.9 | 7.5 | 8.2 | 7.8 | 9.1 | 9.3 | 9.2 |

Combined recovery: Urine + Feces + $CO_2$ - Animal 1, 98.0%; Animal 2, 93.8%; average, 95.9%

[1]N = no feces excreted.
[2]The first ethanolamine sample was taken at 24 hours.

TABLE III

Radiocarbon Residue in Liver and Kidney
as % of Administered Dose

| Hours Post-Treatment | % of Administered Radiocarbon[1] | | | |
|---|---|---|---|---|
| | Liver | | Kidney | |
| | Males | Females | Males | Females |
| 1 | 9.8 | 17.4 | 1.0 | 1.3 |
| 3 | 9.3 | 16.8 | 1.0 | 1.4 |
| 6 | 9.7 | 33.6 | 1.0 | 2.4 |
| 12 | 9.0 | 37.4 | 0.8 | 2.3 |
| 24 | 7.0 | 25.1 | 0.6 | 1.9 |
| 48 | 4.1 | 16.1 | 0.4 | 1.2 |

[1]The data are arithmetic averages of analyses from two animals.

TABLE IV

Radiocarbon Residues in Tissues Ten Days After Administration of A Single Oral Dose of DI-SYSTON-$^{14}$C

| Sample | Ppm - DI-SYSTON Equivalents[1] | |
|---|---|---|
| | Males | Females |
| Liver | 0.154 | 0.119 |
| Kidney | 0.051 | 0.026 |
| Heart | 0.016 | 0.004 |
| Fat | 0.090 | 0.009 |
| Muscle | 0.012 | 0.002 |
| Brain | 0.015 | 0.006 |
| Skin | 0.037 | 0.006 |
| Blood | 0.007 | <0.002 |

[1]The data are arithmetic averages of analyses from two animals.

TABLE V

Quantitative Distribution of DEP and DEPT in Urine

| Hours Post-treatment | DEP and DEPT as % of Urinary Activity | | | | | |
|---|---|---|---|---|---|---|
| | Males | | | Females | | |
| | DEP | DEPT | DEP + DEPT | DEP | DEPT | DEP + DEPT |
| 0 - 4 | 31.9 | 61.1 | 93.0 | 29.1 | 58.9 | 88.0 |
| 4 - 8 | 49.8 | 42.2 | 92.0 | 51.8 | 38.9 | 90.7 |
| 8 - 12 | 70.8 | 23.2 | 94.0 | 62.8 | 30.3 | 93.1 |
| 12 - 24 | 83.7 | 11.3 | 95.0 | 85.1 | 10.0 | 95.1 |
| 0 - 72[1] | 45.3 | 47.8 | 93.1 | 76.7 | 16.8 | 93.5 |

[1]Composite.

TABLE VI

Distribution of DEP and DEPT in Urine

| Hours Post-Treatment | DEP and DEPT as % of Dose | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | | | Females | | | |
| | DEP | DEPT | DEP + DEPT | DEP/DEPT | DEP | DEPT | DEP + DEPT | DEP/DEPT |
| 0 - 4 | 13.3 | 25.4 | 38.7 | 0.52 | 1.8 | 3.7 | 5.5 | 0.49 |
| 4 - 8 | 9.6 | 8.1 | 17.7 | 1.19 | 2.6 | 1.9 | 4.5 | 1.37 |
| 8 - 12 | 4.2 | 1.4 | 5.6 | 3.00 | 7.8 | 3.8 | 11.6 | 2.05 |
| 12 - 24 | 5.8 | 0.8 | 6.6 | 7.25 | 16.9 | 2.0 | 18.9 | 8.45 |
| Totals | | | | | | | | |
| 0 - 24 | 32.9 | 35.7 | 68.6 | 0.92 | 29.1 | 11.4 | 40.5 | 2.55 |
| 0 - 72 (Composite) | 37.0 | 39.0 | 76.0 | 0.95 | 52.4 | 11.5 | 63.9 | 4.56 |

TABLE VII

Distribution of Radiocarbon in Or[illegible]oluble and Watersoluble Fractions of Liver

| Hours Post-Treatment | Distribution as % of Total Liver R[illegible]e[1] | | | | | |
|---|---|---|---|---|---|---|
| | Male | | | [illegible]emale | | |
| | Water | Chloroform | Total | Water | [illegible]croform | Total |
| 1 | 47.8 | 2.4 | 50.2 | 40.5 | [illegible].0 | 41.5 |
| 3 | 39.3 | 3.4 | 42.7 | 25.8 | 1.0 | 26.8 |
| 6 | 45.5 | 2.0 | 47.5 | 33.0 | 0.8 | 33.8 |
| 12 | 43.6 | 1.2 | 44.8 | 37.7 | 0.4 | 38.1 |
| 24 | 17.0 | 1.0 | 18.0 | 18.5 | 0.3 | 18.8 |
| 48 | 34.1 | 0.4 | 34.5 | 35.2 | 0.3 | 35.5 |

[1]Each value is an arithmetic average of analyses from two animals.

TABLE VIII

Distribution of Radiocarbon in Organosoluble and Watersoluble Fractions of Kidney

| Hours Post-Treatment | Distribution as % of Total Kidney Residue[1] | | | | | |
|---|---|---|---|---|---|---|
| | Male | | | Female | | |
| | Water | Chloroform | Total | Water | Chloroform | Total |
| 1 | 76.7 | 5.6 | 82.3 | 67.1 | 4.2 | 71.3 |
| 3 | 78.0 | 4.0 | 82.0 | 77.9 | 1.9 | 79.8 |
| 6 | 75.8 | 3.6 | 79.4 | 74.9 | 0.6 | 75.5 |
| 12 | 61.9 | 1.9 | 63.8 | 67.5 | 0.5 | 68.0 |
| 24 | 56.5 | 1.9 | 58.4 | 56.7 | 0.7 | 57.4 |
| 48 | 41.6 | 1.4 | 43.0 | 38.9 | 0.9 | 39.8 |

[1]Kidneys from two animals were combined prior to extraction.

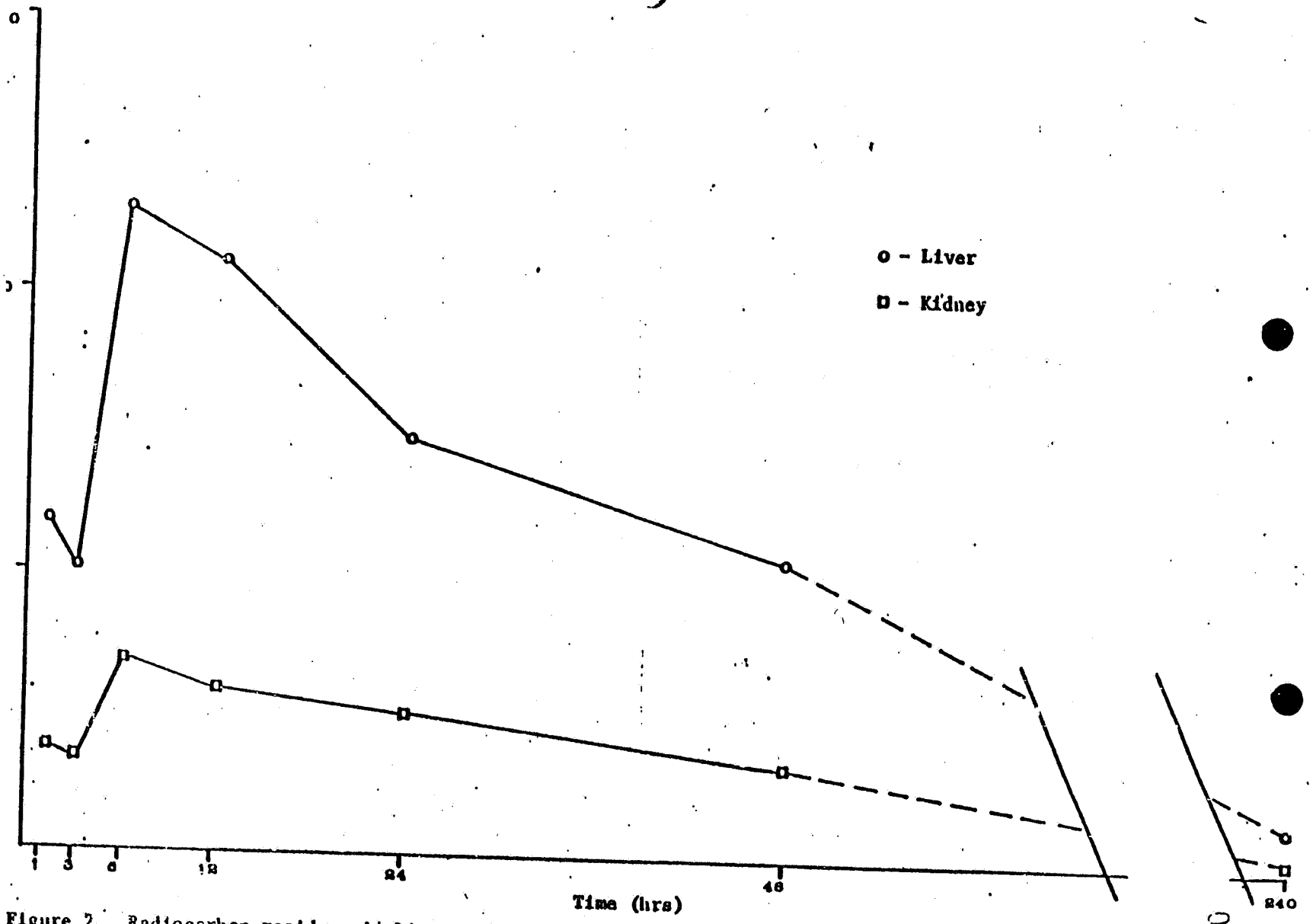
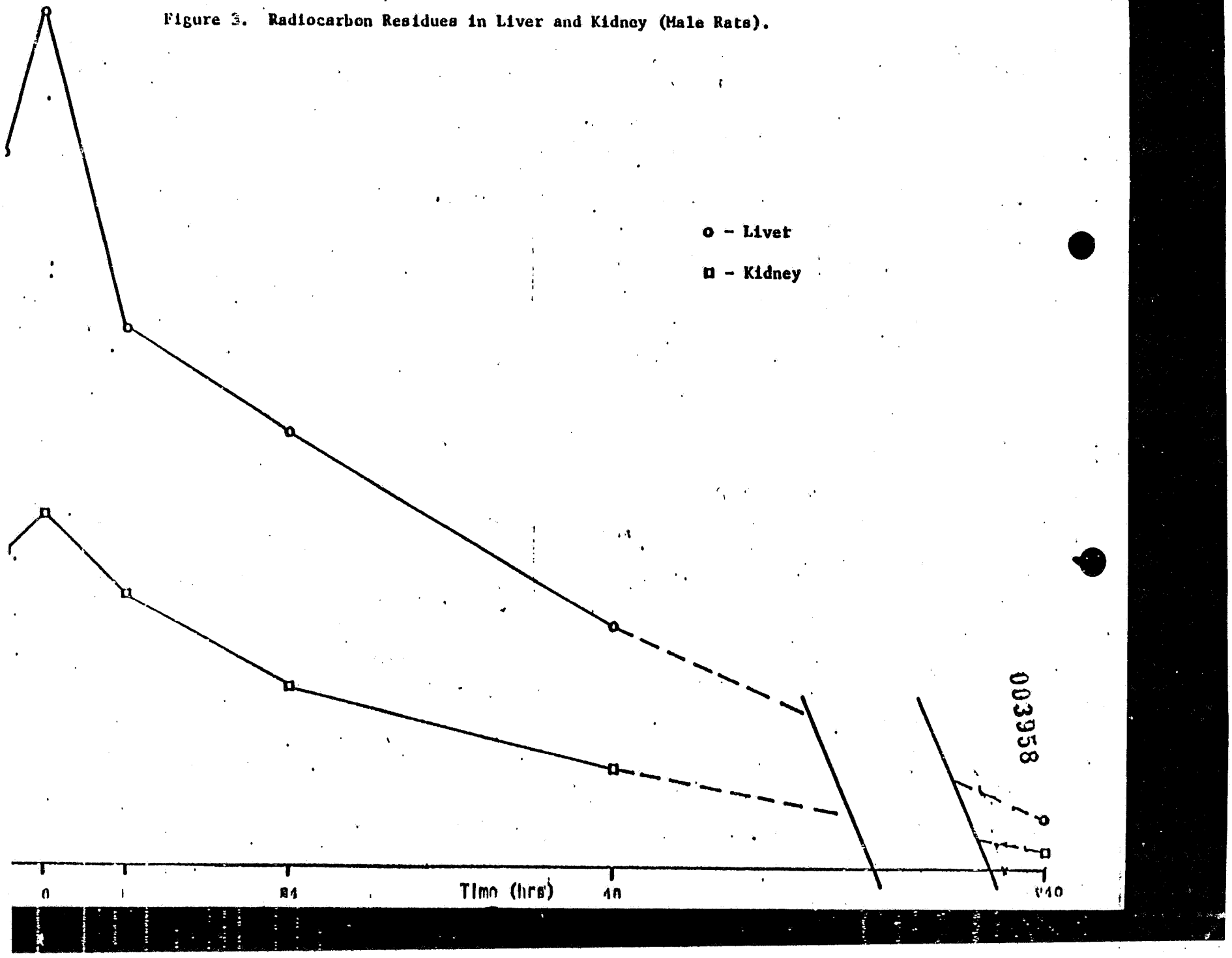
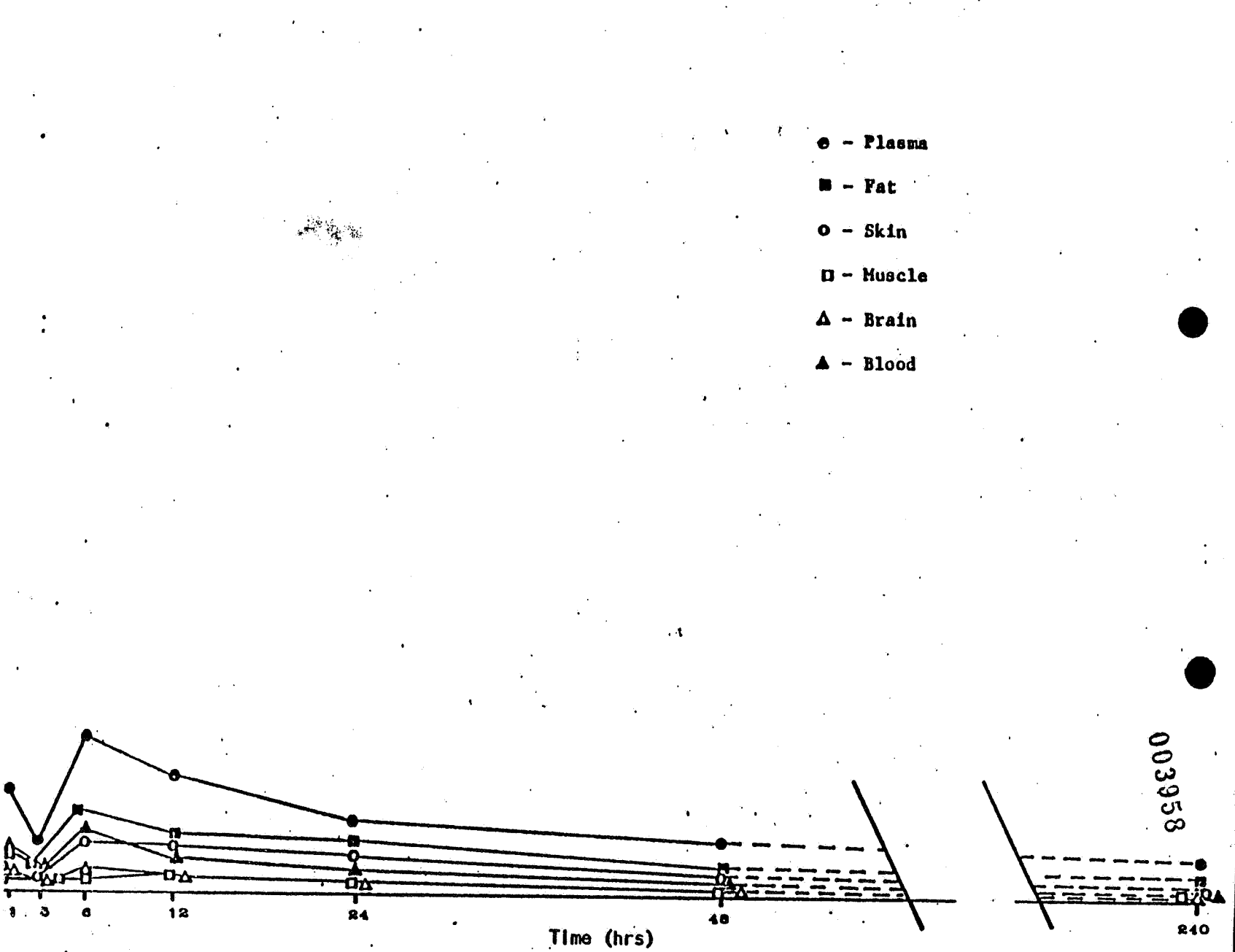


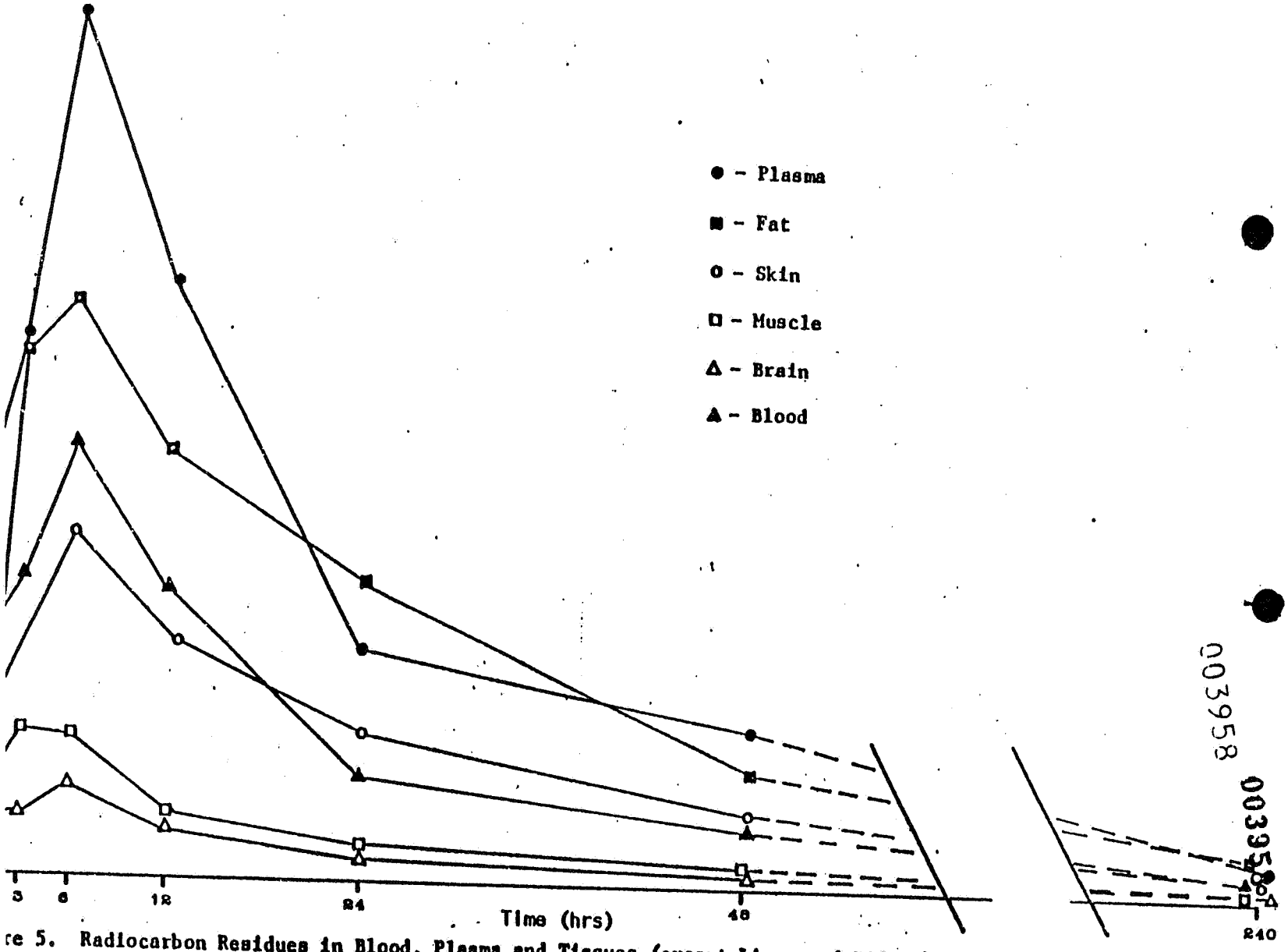

Figure 2. Radiocarbon residues in Liver and Kidney (Female rats)

Figure 3. Radiocarbon Residues in Liver and Kidney (Male Rats).






gure 4. Radiocarbon Residues in Blood, Plasma and Tissues (except Liver and Kidney) - Femal




re 5. Radiocarbon Residues in Blood, Plasma and Tissues (except Liver and Kidney) - Male

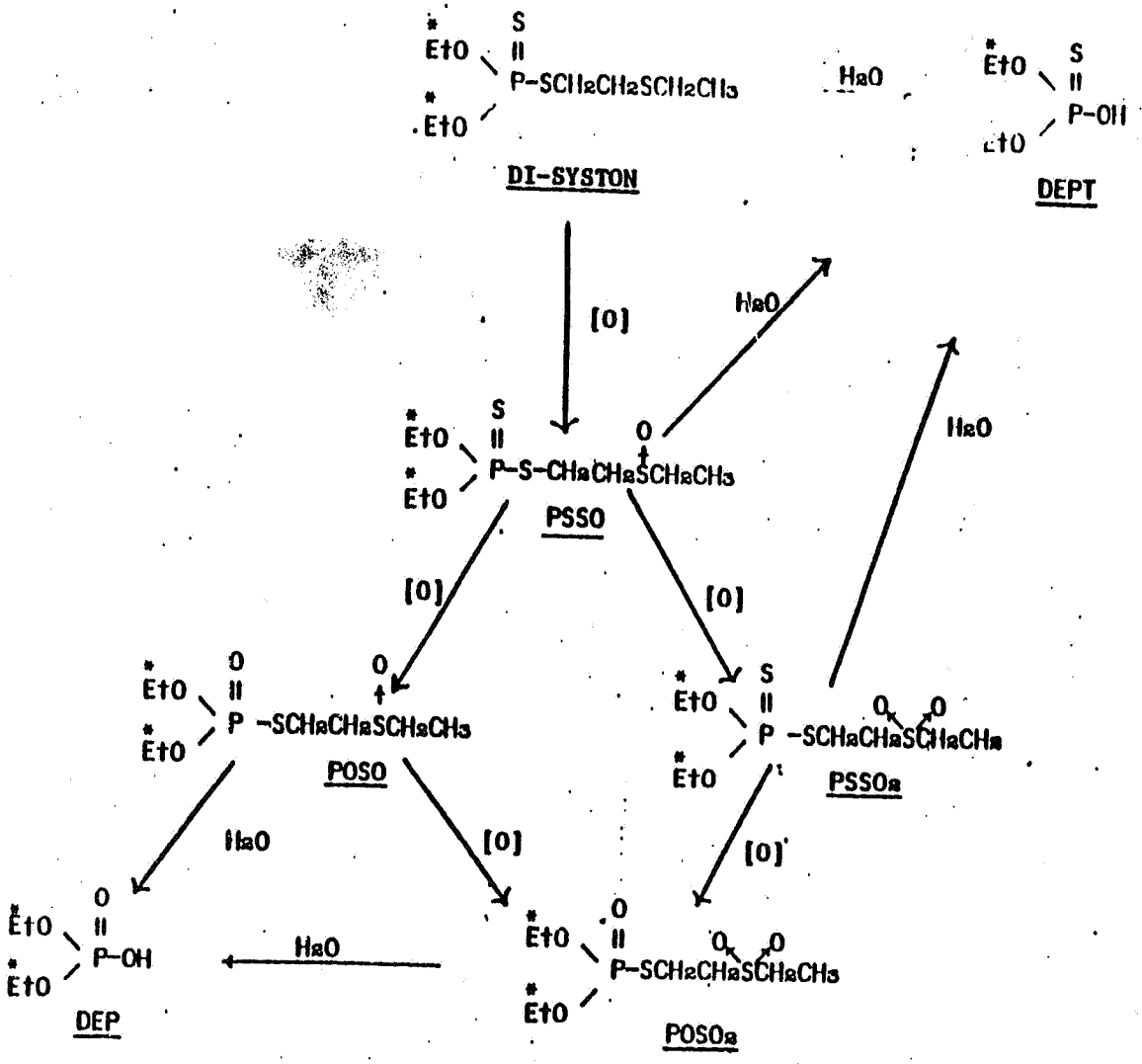
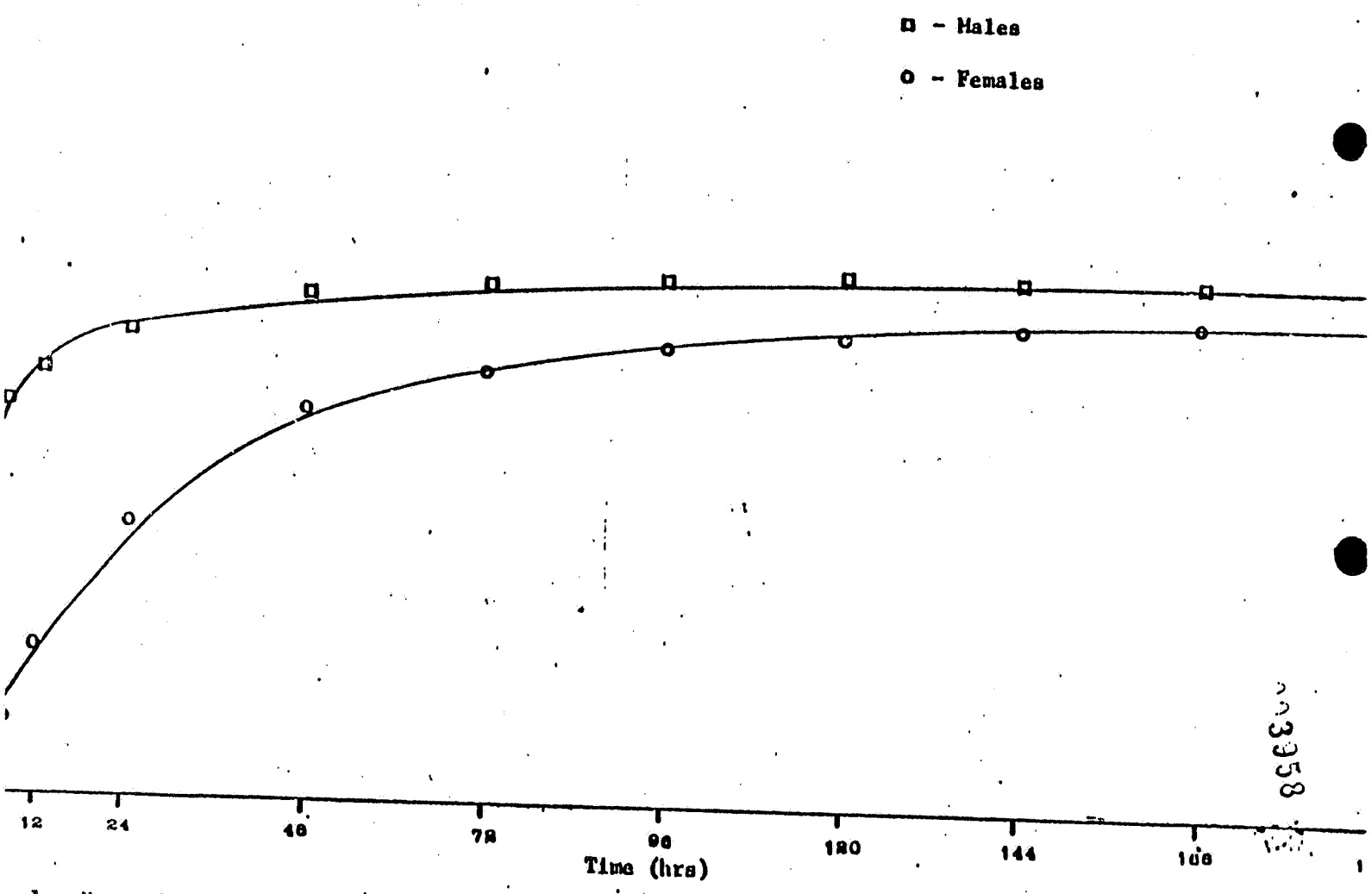



Figure 6. Proposed reaction pathways for metabolism of DI-SYSTON by .




e 1. Excretion of Radiocarbon in Urine following Administration of a Single Oral Dose of DI-SYSTON